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CERCOSPORA LEAFSPOT OF RED BUD

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(WITH 11 FIGURES)

One of the most conspicuous diseases of red bud, *Cercis canadensis* L., within the area comprising the Duke Forest, is a leafspot whose cause is commonly designated *Cercospora cercidicola* Ellis. It appears from the records of collections that this fungus is coextensive in range throughout the eastern United States with that of its host. The writer's interest in this disease has centered, for several years, in the developmental morphology of the pathogen. It has been found that the fungus possesses not only a conidial stage but also an ascigerous stage. The former may be found on living leaves throughout the entire period from April to October, and the latter matures in March on decaying leaves, beginning its development, however, during the preceding autumn, with the formation of spermatia and carpogonia.

CONIDIAL STAGE

Cercospora leafspot can be recognized by the presence of circular to angular rusty-brown to dark-brown necrotic lesions, usually 4–5 mm. in diameter. The border is definite, raised, and dark-brown during the early part of summer, but by autumn large indefinitely limited spots having a diameter of a centimeter or more will have developed. The spots become grayish above but remain rusty-brown beneath. The tissues surrounding the necrotic areas early become yellowish-green (FIG. 11).

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The conidiophores are produced on both leaf surfaces but are much more abundant on the lower surface. They emerge through the stomates and occur for the most part in clusters of 5 to 10. They generally adhere at the base, by one-third to one-half their length, and the upper portion spreads into a loose fascicle (FIGS. 1, 2). In some cases each conidiophore of the fascicle tends to stand apart from each of the others. They are more or less branched, have prominent denticulate conidial scars, and vary in dimensions from $50–300 \times 3–5\ \mu$. The conidia (FIG. 4) are obclavate, straight or curved, dilutely colored, 1–3-septate, and 15–50 (mostly $25–30\ \mu$) $\times\ 4–6.5\ \mu$. Conidia continue to be formed throughout the entire summer whenever moisture conditions are favorable, and meantime the stromata at the bases of the conidiophoral fascicles increase in size.

This stage of the fungus was first collected near Lexington, Kentucky, by W. A. Kellerman and was described by J. B. Ellis (**1**), in 1882, as *Cercospora cercidicola.* The organism is also included among species of *Cercospora* enumerated by Ellis and Everhart (**3**). Tehon (**5**), in 1924, erected the name *Cercospora cercidicola* var. *coremioides,* mainly because the conidiophores are closely adherent throughout a great part of their length. Solheim (**4**) expressed the opinion in his monograph that this varietal name should be relegated to synonomy since the organism is characteristically coremioid. With this opinion the present writer is entirely in accord.

PERITHECIAL STAGE

At about the time that the leaves are being shed the lower surface of lesions may be observed to be densely occupied by dark erumpent stromata. These stromata are of two types, the spermogonial initials and the perithecial primordia, structures that can be distinguished if infected tissues are appropriately fixed, sectioned and stained. Both are spherical with a diameter ranging from 50 to $80\ \mu$. As they continue development the parietal portion of each, consisting of several layers of brown, thick-walled cells, becomes differentiated. The spermogonia eventually become pycnidium-like, the interior coming to be filled with bacilliform spermatia (FIG. 6). These spermatia are embedded in a gelatinous matrix

that arises from the disintegration of the spermatiferous cells. There is evidence that spermatia are first formed near the center of the spermogonial stroma and that formation of spermatia proceeds centrifugally. As soon as the wall of the perithecial pri-

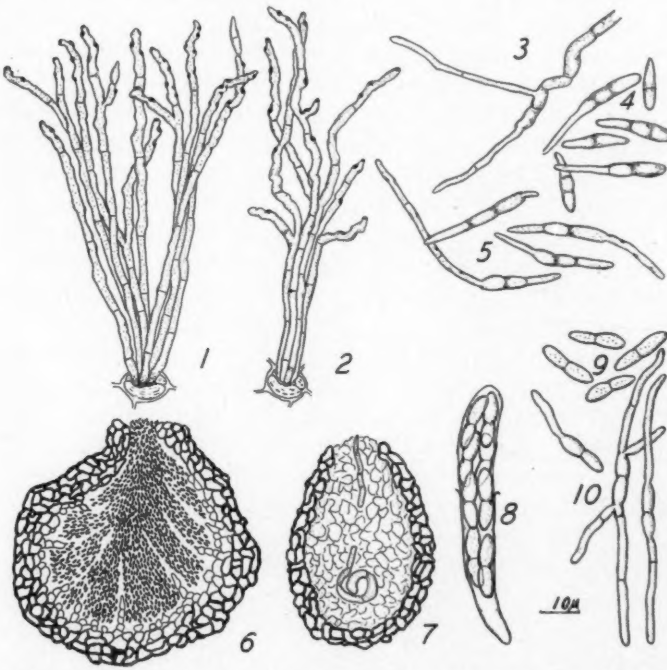

FIG. 1, loose fascicle of conidiophores of *Cercospora cercidicola;* 2, coremoid fascicle of conidiophores; 3, germinating conidiophore; 4, conidia; 5, germinating conidia; 6, mature spermagonium in vertical section; 7, perithecial primordium, in section, containing coiled archicarp with trichogne extending to the surface of the leaf; 8, ascus of *Mycosphaerella cercidicola* whose outer membrane is ruptured, and the inner membrane is extended preparatory to ascospore ejection; 9, mature ascospores; 10, germinating ascospores.

mordia has differentiated the interior will be found to be filled with cells having a deeply staining content. Moreover within each is a single coiled archicarpic apparatus whose trichogynal portion projects to the surface (FIG. 7). Although spermatiation was not

observed, it undoubtedly is accomplished with films of moisture serving as the medium of transfer of spermatia. Then by late March or early April of the succeeding spring the perithecial primordia will have become transformed into mature perithecia.

The perithecia are densely aggregated within the areas occupied by lesions during the previous year. They are completely embedded, except for the ostiolar orifices, within the leaf tissues. Nearly all open to the lower leaf surface. They range in diameter from 65–95 μ. The asci adhere in a fascicle when the perithecia are crushed in a drop of water. They measure $35–45 \times 7–8\ \mu$. Their outer wall ruptures and the asci become elongated preparatory to discharge of the ascospores (FIG. 8). The ascospores are unequally two-celled, constricted at the septum, slightly curved, and measure $15–18 \times 4–5\ \mu$ (FIG. 9). Paraphyses are absent. These morphological features are clearly those that characterize the genus *Mycosphaerella.*

Apparently two species of *Mycosphaerella* (*Sphaerella*) are known to occur on *Cercis, S. Cercidis* Pass., and *S. cercidicola* Ellis & Kellerm. The former was described from Italy, occurring there on *Cercis japonica* Sieb., and is not known to occur in North America. The latter was first collected in Kansas in June, 1884, by W. A. Kellerman, who sent specimens to Ellis. Later in the year Ellis and Kellerman (3) described it as *S. cercidicola,* but their description was not included in Saccardo's Sylloge Fungorum. The writer has examined their type specimens, No. *550,* deposited in the New York Botanical Garden [1] and found them to be specifically identical with the fungus herein under consideration. Two discrepancies appear in connection with Ellis and Kellerman's (3) description. In the first place the perithecia are said to be mostly epiphyllous whereas they are found to be mostly hypophyllous. Secondly the ascospores are recorded as measuring $11–13 \times 2.5–3.0\ \mu$, but are $15–18 \times 4–5\ \mu$. The writer's measurements were made of freshly discharged ascospores, while Ellis and Kellerman probably made measurements from dried specimens collected a few months previously. Furthermore the ascospores of certain species of *Mycosphaerella* are known to enlarge considerably immediately

[1] Thanks are due Dr. F. J. Seaver, New York Botanical Garden, for courtesies in connection with my examination of specimens in the Herbarium.

prior to discharge, and the asci from dried specimens of such species never contain mature ascospores.

GROWTH IN CULTURE

The fungus has been isolated in pure culture from conidia and from ascospores. The cultures originating from conidia were obtained by making a suspension of conidia and streaking loopfuls of the suspension over the surface of Petri dishes containing hardened potato nutrient agar. The cultures from ascospores were obtained by permitting the ascospores to be forcibly ejected onto the surface of poured agar plates. To do this, leaves bearing mature perithecia were placed in the tops of Petri dishes and the agar in the bottoms were inverted above the perithecia.

The appearance of germinating conidia (FIG. 5) and of ascospores (FIG. 10) in the early stages of colony formation resembled that of various other species of *Cercospora* and *Mycosphaerella* which the writer has studied in culture. Growth is slow, three or four months being required to produce a colony one cm. in diameter. The colonies are white to pale-brown in color, occasional ones being olivaceous-black. They are compact, elevated, almost hemispherical, and the surface is cerebroid. It is impossible at any time to distinguish cultures originating from ascospores from those originating from conidia.

The conidiophores of this fungus germinate, forming hyphae (FIG. 3), under conditions favorable for the growth of conidia. Although hyphal formation by conidiophores may be anticipated to occur, it is not a common phenomenon among the Fungi Imperfecti.

PATHOGENICITY

Since the pure cultures remained sterile, the tests for pathogenicity were limited to the use of crude inoculum. Conidial suspensions, made by washing lesions, were applied to healthy foliage with the result that characteristic leafspots appeared two to three weeks after inoculation. When decaying leaves, bearing perithecia, were attached to healthy leaves, similar leafspots developed within three weeks. The lesions resulting from each kind of inoculum, bore conidiophores and conidia typical of *Cercospora cercidicola*. There appears to be no doubt from the infection experiments that

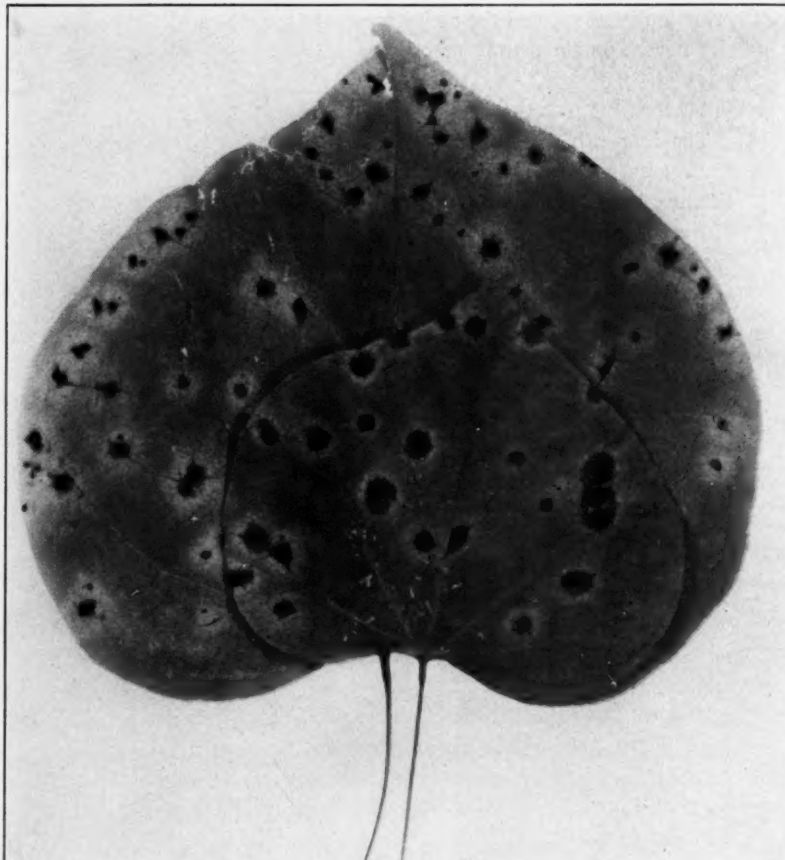

FIG. 11. Red bud leaf bearing lesions produced by action of the pathogen, *M. cercidicola.*

the fungus is pathogenic, and that the conidial and perithecial stages are genetically connected.

TAXONOMY

The following brief description of the red bud pathogen has been prepared since this study connects, for the first time, the conidial stage and the hibernating or perithecial stage, and since the fungus has previously not been correctly and adequately described.

Mycosphaerella cercidicola (Ellis & Kellerm.) comb. nov. emend.

Perithecia in vernale in foliis putrescentibus efformantia, vulgo hypophylla interdum epiphylla, in maculis dense dispersa, punctiformia, nigra, erumpenti-immersa, sphaeroidea, 65–95 μ diam.; ascis sacciformibus, fasciculatis, aparaphysatis, octosporis, 35–45 × 7–8 μ; sporidiis sub-biseriatis, loculis inaequalis, loculo superiore crassiore constrictis, rectis vel curvulis, 15–18 × 4–5 μ.

Spermogoniis et carpogoniis autumno efformantibus, innatis, paginis inferioribus in maculis exaridis occupantibus, globosis, nigris, 50–80 μ; spermatiis bacillaribus, hyalinis, 2–3 × 1 μ. Hab. in foliis maturissimis vel dejectis Cercidis.

Status conidicus: Statum conidicum Cercospora cercidicola Ellis sistit. Maculis initio minutis, dein expansis 3–4 mm., subcircularibus v. augulosis, denique 1 cm., subnigricantibus, denique supra griseo-albidis, infra vero rubiginosis, linea nigro-brunnea subelevata circumicirca rufo-zonata cinctis; hyphis amphiginis, laxe fasciculatis, brunneis, 50–300 × 3–5 μ, geniculatis; conidiis oblongo-clavatis, tenuiter 3-septatis, 13–50 × 4–6.5 μ.

Hab. in foliis vivis *Cercidis canadensis* L., *C. Japonicae* Sieb., *C. occidentalis* Torr.

Syn. *Sphaerella cercidicola* Ellis & Kellerm., Bull. Torrey Club **11**: 123. 1884.

Cercospora cercidicola Ellis, Am. Nat. **16**: 810. 1882.

Cercospora cercidicola var. *coremioides* Tehon, Mycologia **16**: 140. 1924.

Specimens of both the conidial and ascigerous stages have been deposited in the Farlow Herbarium, Harvard University, the Mycological Collections, U. S. Department of Agriculture, and the herbarium of the New York Botanical Garden.

SUMMARY

The developmental cycle of the fungus generally known as *Cercospora cercidicola* Ellis, causing a leafspot of red bud, has been studied. As a result it has been found that, in addition to the conidial stage, the pathogen possesses a perithecial stage. This perithecial stages proves to be *Mycosphaerella cercidicola* (Ellis & Kellerm.) Wolf.

The conidial stage is parasitic.

The spermogonia and carpogonia, that initiate the perithecia, are developed in late summer and early autumn.

The perithecial stage matures on decaying leaves in the following spring.

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5. **Tehon, L. R.** Notes on the parasitic fungi of Illinois. Mycologia **16**: 135–142. 1924 (see p. 140).

DASYSCYPHAE ON CONIFERS IN NORTH AMERICA. IV. TWO NEW SPECIES ON DOUGLAS FIR FROM THE PACIFIC COAST

Glenn Gardner Hahn

(with 2 figures)

INTRODUCTION

As part of the investigation of the European larch-canker parasite, *Dasyscypha Willkommii* (Hart.) Rehm (**2**, **5**, **6**), introduced from Great Britain into Massachusetts, Dasyscyphae on native and planted Douglas fir [*Pseudotsuga taxifolia* (La M.) Britt.] in North America had to be distinguished. This taxonomic research was necessary because Douglas fir in Europe had been reported attacked by the larch-canker organism (**5**, p. 900), and Douglas fir in New England was affected by a canker (**5**, *fig. 4*) with which a *Dasyscypha* was associated. It thus became important to determine the relationship of the Dasyscyphae occurring on this economically important conifer. Two species on Douglas fir, the introduced European saprophyte, *D. calycina* Fuckel (nec *Peziza calycina* Schum.) (**2**), and the native organism, *D. Ellisiana* (Rehm) Sacc. (**3**), a species associated with a resinous canker of planted blue Douglas fir along the Atlantic Seaboard (**3**; **5**, pp. 903–904), have already been reported. This paper is concerned with two hitherto undescribed *Dasyscypha* species restricted in their habitat as far as known to Douglas fir growing on the Pacific Coast.

TWO NEW DASYSCYPHAE ON DOUGLAS FIR

In 1930 members of the Division of Forest Pathology discovered a conspicuous open canker (fig. 1 : 1) associated with a species of *Dasyscypha* on saplings and poles of Douglas fir, suppressed or growing on poor sites, in certain areas in the West. The type of canker upon which the undescribed *Dasyscypha* (**1**, p. 263) was

growing is somewhat similar to that produced on larch by *Dasyscypha Willkommii* (**2**, **5**). At approximately the same time a saprophytic *Dasyscypha* also was collected on small twigs of Douglas fir in the Pacific Northwest. The western Dasyscyphae are herein described as new species.

Dasyscypha Pseudotsugae sp. nov.

Apothecia, waxy, fleshy, sparse, scattered or closely grouped (FIG. 1 : 2), erumpent, at first globular, closed, opening in a roundish form, margin incurved, urn-like, becoming widely expanded, disc-like under moist conditions, when dry laterally compressed and closed, shortly but distinctly stipitate, externally whitish; disc light orange-yellow [1] to orange, 1.0–3.5 mm. diam.; excipular hairs persistent, minutely roughened, hyaline, thin-walled, cylindrical with obtusely rounded extremities, septate, cells short, 3.0–3.5 μ broad. Asci clavate, apex obtusely rounded, range (50) 47.0–60.0 × 3.4–5.4 μ. Ascospores eight, uniseriate, arranged in a regular oblique manner, hyaline, smooth, continuous both in the ascus and upon germination, elliptical or elliptic-fusiform with obtuse extremities, range (100) 3.8–7.2 × 1.8–3.6 μ. Paraphyses outranking asci, filiform, of even diameter, septate, not swollen or only slightly so toward the tip with obtuse extremities, minutely guttulate.

Conidial stage (FIG. 1 : 4) consisting of waxy, fleshy, erumpent, light-buff stromata, with irregular labyrinthiform cavities in which the conidia are borne, and from which they exude in an opaque droplet or tendril; conidia abstricted from the tips of short, slender, subulate conidiophores, extremities acute, simple or verticillately branched; conidia hyaline, continuous, elliptic, extremities obtuse, range (50) 3.4–4.0 × 2.4–3.0 μ, conidia artificially produced on malt agar, range (50) 2.4–4.0 × 1.8–3.0 μ. Germination observed.

Ascomatibus sparsis, solitariis vel gregariis, initio subglobosis, dein cyathiformibus, cupulis humidulis planiusculis, margine in sicco semper connivente, breviter stipitatis, extus albidis, tomentosis; disco luteo-aurantiaco vel aurantiaco, 1.0–3.5 mm. diam.; pilis, brevibus, hyalinis, septatis, minute asperatis, 3.0–3.5 μ crassis. Ascis octosporis, clavatis, subcylindraceis (50) 47.0–60.0 × 3.4–5.4 μ. Ascosporis monostichis, continuis, hyalinis, ellipsoideis vel ellipsoideo-fusiformis, apice obtusis, (100) 3.8–7.2 × 1.8–3.4 μ. Paraphysibus filiformibus, septatis, ascos superantibus, apice obtusis non vel leniter sursum incrassatulis, guttulatis.

Fructificationibus conidicis luteis, carnosis, ceraceis, erumpentibus, loculiis

[1] The color nomenclature used is that of R. Ridgway, Color standards and color nomenclature. 1922. Washington, D. C.

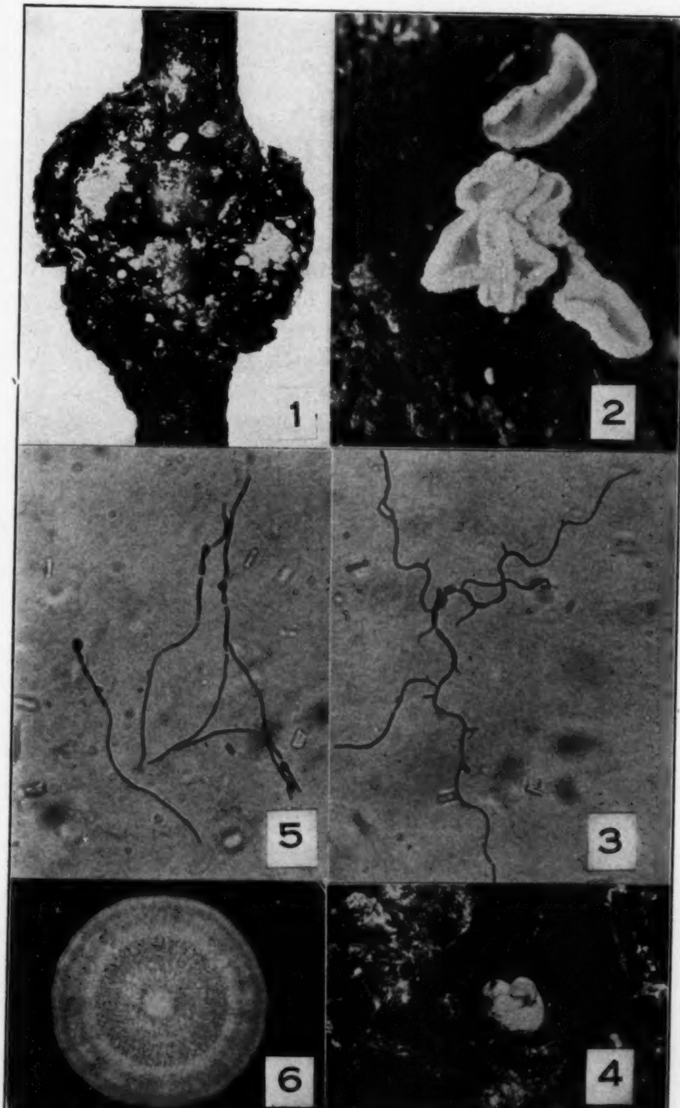

FIG. 1. *Dasyscypha Pseudotsugae.*

labyrinthiformibus; conidiophoris brevibus, hyalinis, filiformibus, simplicibus vel verticillate ramosis; conidiis hyalinis, continuis, ellipsoideis, 3.4–4.0 × 2.4–3.0 μ crassis.

Hab. in ramulorum et truncorum viventium partibus aegrotis *Pseudotsugae taxifoliae* in *America Boreali*. Specimen typicum *68168*,[2] *P. taxifolia*, in Mycological Collections, Bureau Plant Industry, U. S. D. A., Washington, D. C.

HABIT. The type specimen growing on cankers collected from living branches of *Pseudotsuga taxifolia*, Lokoya, Napa County, California, by J. L. Mielke, *68168*, has been deposited in the Mycological Collections, Bureau Plant Industry.

Other specimens of the fungus collected on living branches and trunks of *P. taxifolia* from 1930 to 1939 and deposited in the collections of the Division of Forest Pathology have been studied.

California. *53062*, near Hayfork, Trinity County, coll. J. S. Boyce; *53063*, near Arcadia, Napa Co., J. S. B.; *53066*, Alder Springs, Glenn County, coll. J. S. B.; *53084*, Lokoya, Napa Co., coll. J. S. B.; *53103–4–5*, Lokoya, coll. W. W. Wagener and G. G. Hahn; *64014*, Dry Creek Road, Napa Co., coll. J. S. B.; *64016*, Triniti, Sonoma Co., coll. J. S. B.; *64017*, Hutchinson Ranch south of Santa Rosa, Sonoma Co., coll. J. S. B.; *64018*, Coit Estate, near Larkmead, Napa Co., coll. J. S. B.; *68169*, Siskiyou National Forest, coll. J. L. Mielke; *68170*, Lokoya, coll. J. L. M.

Oregon. *40538*, near Maryland College, Oswego, coll. J. R. Hansbrough; *40547*, near Oakridge, coll. H. G. Lachmund and J. S. B.; *40591–92*, near Zig Zag Ranger Station, Mt. Hood Nat. For., coll. J. R. H.; *40593–95–96*, Zig Zag Ranger Sta., coll. T. W. Childs, J. L. M. and J. R. H.; *40597*, near Kingston, coll. J. L. M. and T. W. Childs; *40599–600*, near Falls City, coll. T. W. C.; *40672*, Logie Trail near Portland, coll. T. W. C. and J. R. H.; *40680*, Wolf Creek near Rosebery, coll. L. N. Goodding; *64015*, Wolf Creek, Josephine Co., coll. J. S. B.

British Columbia. *40500*, D'Arcy, near Anderson Lake Lodge, coll. J. R. H.

Dasyscypha Pseudotsugae is most closely related to *D. calyciformis* (Willd.) Rehm, but differs from the European species, which occurs principally on *Abies*, but also on *Pinus*, *Picea*, and

[2] Unless otherwise indicated, collection numbers denote specimens for study filed in the Division of Forest Pathology, New Haven, Conn.

Larix, in its host relationship and spore characters. *D. calyciformis* is most commonly found as a saprophyte but in some cases it is associated with canker formations, and accordingly has been regarded as being parasitic. The imperfect stage of *D. calyciformis* is nonconspicuous and its conidia germinate with great difficulty (**7**, pp. 15–19). On the other hand the conidia of *D. Pseudotsugae* occur abundantly and are readily germinable.

A pathological investigation has not been undertaken to determine whether the pronounced cankers on living trees commonly associated with *Dasyscypha Pseudotsugae* are caused by that fungus. Field studies on the disease have been made by different members of the Division of Forest Pathology including Boyce (**1**, p. 263), who discussed the canker in his text together with his observations on the disease in the known northern range of *D. Pseudotsugae,* as well as in its southern range. In California and Oregon the canker is commonly found on the main trunks and branches of small saplings. In the north the fungus occurs also on roughened bark, particularly about pruning wounds, on the trunks of trees of pole size up to 8 inches in diameter.

Based on present observations, *Dasyscypha Pseudotsugae* and the canker associated with it appear to have little pathological importance. However it still remains to be seen how the organism may behave in the future. In a pathological investigation of the disease, we shall need to discover the role of not only the perfect stage, but also that of the imperfect stage. In this instance we have two types of spores both of which are probably capable of disseminating the canker, for, as stated above, the new species, unlike the European larch-canker organism, produces functionable conidia. No one has succeeded in growing *D. Willkommii* from the spores produced by its imperfect stage. Apparently the conidia play no part in the dissemination of the European canker.

Dasyscypha ciliata sp. nov.

Apothecia (FIG. 2: 1), waxy, fleshy, usually scattered, occasionally grouped (FIG. 2: 2), at first globular, closed, opening as a flat disc under moist conditions, laterally compressed and closed when dry, very shortly but definitely stipitate, externally whitish; disc

orange, commonly 1–2 mm. diam.; excipular hairs conspicuous, elongate, minutely roughened, hyaline, thin-walled, cylindrical with subacute extremities, brittle, readily breaking away and revealing the glabrous tissue beneath, hairs about the rim persistent, fringe-like, septate, cells short, 3 μ broad. Asci (FIG. 2: 3), clavate, apex obtusely rounded, range (150) 63.0–92.8 × 6.0–12.0 μ, commonly 70–80 × 7–10 μ. Ascospores eight, uniseriate, arranged in a regular oblique manner, hyaline, smooth, continuous in ascus and upon germination, with a prominent guttule (FIG. 2: 3), ovate or elliptic with obtuse extremities, range (100) 8.0–12.4 × 4.0–6.6 μ. Paraphyses outranking asci (FIG. 2: 5), filiform, septate, of equal diameter and unswollen or very slightly so at the tips.

Conidial stage not observed either in nature or in pure cultures.

Ascomatibus sparsis, solitariis vel gregariis, erumpentibus, initio subglobosis, dein cyathiformibus, cupulis humidulis planiusculis, margine in sicco semper connivente, breviter stipitatis, extus primitus distincte albidis, tomentosis, ciliatis, demum nudis, glabris; disco aurantiaco, vulgo 1–2 mm. diam.; pilis distincte elongatis, hyalinis, septatis, minute asperatis, 3 μ crassis. Ascis octosporis, clavatis, subcylindraceis, (150) 63.0–92.8 × 6.0–12.0 μ, vulgo 70–80 × 7–10 μ. Ascosporis monostichis, continuis, hyalinis, ovatis, (100) 8.0–12.4 × 4.0–6.6 μ. Paraphysibus filiformibus, septatis, ascos superantibus, apice obtusis non sursum incrassatulis, guttulatis.

Fructificationibus conidicis non visis.

Hab. in ramulis emortuis *Pseudotsugae taxifoliae* in America Boreali. Specimen typicum *68062, P. taxifolia,* in Mycological Collections, Bureau Plant Industry, U. S. D. A., Washington, D. C.

HABIT. The type specimen, *68062,* collected on dead branchlets or small branches of *Pseudotsuga taxifolia,* Portland Heights, Oregon, by J. R. Hansbrough, April 21, 1931, has been deposited in the Mycological Collections, Bureau Plant Industry.

Other specimens of the fungus on Douglas fir collected from 1930 to 1939 and deposited in the collections of the Division of Forest Pathology have been studied:

Oregon. *53094*, Rhododendron, coll. G. G. Hahn; *68033*, Rhododendron, coll. J. W. Kimmey; *68061*, Rhododendron, coll. J. W. K. and T. W. Childs; K.100, Hood River, coll. J. E. Kienholz (Herb. J. E. K.).

British Columbia. *40502,* Revelstoke, coll. J. R. Hansbrough; 40516, Owl Creek, coll. J. R. H.; *40531–32* Hunters Siding (Rosebery), coll. J. R. H.; *58002,* Revelstoke, coll. J. L. Mielke.

This new species should not be mistaken for *Dasyscypha Agassizii* (Berk. & Curt.) Sacc. (**8**) with which it might be confused.

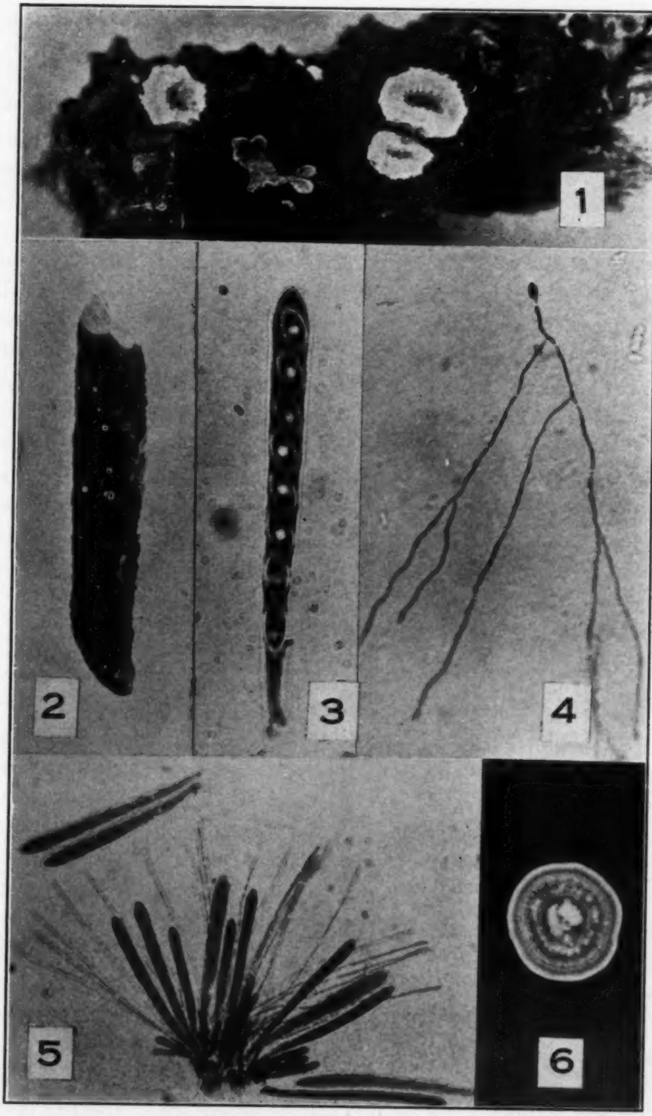

FIG. 2. *Dasyscypha ciliata.*

It differs from the latter species, which is found commonly in the eastern United States on balsam fir [*Abies balsamea* (L.) Mill.] and northern white pine (*Pinus strobus* L.), in spore characters and in the size of its fruit cups. An imperfect stage which is present in the life-history of *D. Agassizii* is apparently lacking in the life-history of this new Douglas fir species. In this, *D. ciliata* resembles the pine parasite, *D. Pini,* in whose life-history an imperfect stage has not been observed (**4**).

Dasyscypha ciliata is a saprophyte which is fairly uncommon, at least not present abundantly in the areas where it occurs. Unlike *D. Agassizii,* which grows not only on small branches, but also on large trunks, so far as we know *D. ciliata* is restricted in its growth to the small, shaded-out, dead branches and twigs of Douglas fir. In this respect, the saprophyte resembles the pine parasite, *Atropellis pinicola* Zeller & Goodding (Phytopathology **20**: 555–567. 1930), and the European parasite of silver fir, *Phomopsis abietina* (Hart.) Wilson & Hahn (Brit. Myc. Soc. **13**: 261–278. 1938), which likewise are confined to small parts. Investigation may show that certain of the factors influencing restricted colonization of both the saprophyte and the parasites, are identical.

CULTURE NOTES

Both of the new species described in this article grew slowly on 3 per cent synthetic malt agar, *Dasyscypha ciliata* being the slower. Ascospores of both species germinated readily on that medium within 24 hours and did not become uniseptate as did those of the large-spore Dasyscyphae (**2**, **4**). It was found that *D. Pseudotsugae* could be maintained in culture without transfer for six months or more. *D. ciliata* on the other hand was found to stale in culture, and if it was not renewed by subculturing every third month, the colonies failed to grow when placed on a fresh malt-agar substratum.

Dasyscypha Pseudotsugae. Germination of the ascospores was generally bipolar (FIG. 1 : 3) and took place within 24 hours. The initial characters of pure cultures of this species were very similar to those formed by *D. ciliata* on the same medium. On a German malt agar (**7**, p. 92) conidial stromata were formed within a week

in cultures made both from the inner diseased bark of the canker and from monoascus and -ascospore isolations. These conidial fructifications were also produced by the fungus growing in pure culture on sterilized Douglas fir twigs. The spores oozed from the pycnidia in opaque droplets or in light buff or pinkish buff spore-horns. Unlike those of the Dasyscyphae hitherto reported (**2**, **3**, **4**), the imperfect stage of *D. Pseudotsugae* is germinable. Germination also occurred within 24 hours and was both bipolar and monopolar (FIG. 1 : 5). Monoascus and -ascospore isolations of the species made September 13, 1930, which by 1938 had been subcultured 15 times, continued to produce germinable conidia. The writer found that these conidia, although somewhat desiccated after being kept in a culture tube for 10 months, germinated and produced culture characters identical with those obtained in fresh 1939 isolations of the perfect stage of the species. The fungus grew most readily and made a considerable whitish aerial growth on Douglas fir twigs. On synthetic malt agar, 2 month-old-monoascospore colonies attained a diameter of 3.5 cm. These colonies were distinctly zonate, roundish with even periphery; the aerial hyphae, fine, low growing, flocculent about the center, whitish becoming tinged with light-buff. In the substratum a warm buff color appeared and a tawny color formed below the inoculum (FIG. 1 : 6).

Dasyscypha ciliata. Germination was generally monopolar (FIG. 2: 4) although the bipolar type was occasionally observed. A roundish compact pompon-like colony consisting of fine, white, silky, aerial, low-growing hyphae formed slowly about the inoculum on the malt-agar slant. After one month roundish monoascospore colonies attained a diameter of 8 mm. These colonies had an even periphery and were indistinctly zonate. In two months the diameter enlarged to 18 mm. The whitish, aerial hyphae became tinged with light- to warm buff and in the substratum a burnt sienna or mahogany-red color was produced throughout (FIG. 2: 6). Conidial stromata were not observed in malt-agar cultures or on sterilized Douglas fir twigs.

SUMMARY

This paper presents the descriptions of two new species, *Dasyscypha Pseudotsugae* Hahn and *D. ciliata* Hahn on Douglas fir from the Pacific Coast.

Dasyscypha Pseudotsugae is a species definitely associated with cankers and roughened bark occurring on the living trunks and branches of saplings and trees of pole-size. The fungus occurs on Douglas fir, suppressed or growing on poor sites, in certain areas from California to British Columbia. The symptomatology of the Douglas fir canker is somewhat similar to that of the European larch canker.

Dasyscypha Pseudsotsugae has a conspicuous imperfect stage. The fungus is culturable from conidia which germinate readily within 24 hours. In this the species differs from the canker parasite, *D. Willkommii* on larch, for no one has succeeded in growing the latter from conidia.

Dasyscypha ciliata is a saprophyte occurring in the Pacific Northwest. Its growth is restricted to shaded-out, dead branches and twigs of small diameter. As far as known an imperfect stage is lacking in its life-history.

Culture notes dealing with the two new species are given.

For the photographs illustrating this article, I am indebted to Mr. C. K. Goodling, Division of Forest Pathology.

DIVISION OF FOREST PATHOLOGY,
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EXPLANATION OF FIGURES

FIG. 1. *Dasyscypha Pseudotsugae* Hahn. 1, Typical canker on branch of suppressed Douglas fir sapling, collected at Lokoya, Napa County, California. Apothecia and imperfect stage are shown growing on diseased tissue. Approx. × 1.5; 2, Habit, on canker. Approx. × 10; 3, Mycelium produced in three days by a germinating ascospore on malt agar. Typical bipolar germination and the original ascospore at the center of the colony, are shown. Approx. × 344; 4, Conidial fructification with a droplet of exuding spores. Approx. × 10; 5, Five conidia on surface of malt agar, three days after germination. Both bipolar and monopolar germination types are shown. Approx. × 344; 6, Two-month-old monoascospore plate culture on malt agar. Nat. size.

FIG. 2. *Dasyscypha ciliata* Hahn. 1, Habit, on bark of dead branchlet of *Pseudotsuga taxifolia.* Approx. × 10; 2, Habit, both single and clustered apothecia. Nat. size; 3, Ascus with ascospores showing prominent guttules. Approx. × 800; 4, Mycelium produced in three days by germinating ascospore. Germination typically monopolar. Approx. × 344; 5, Asci and paaphyses. Approx. × 344; 6, Two-month-old monoascospore plate culture on malt agar. Nat. size.

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THE OCCURRENCE OF FIVE SUCCESSIVE SWARMING STAGES IN A NON-SEXUAL ACHLYA[1]

S. B. SALVIN[2]

(WITH 1 FIGURE)

Diplanetism in the Saprolegniaceae was described for the first time in 1869 by Leitgeb (**6**), when he reported that in his genus *Diplanes* (now known as *Saprolegnia*) the zoöspores escaped from the sporangium as primary, pear-shaped, terminally biflagellate entities, swarmed for a period as such, encysted, either germinated by forming a hypha of germination or emitted a zoöspore of different structure—namely, reniform, laterally biflagellate—which in turn swarmed, encysted, and then gave rise to a germ tube.

The concept of the genus *Achlya*, originally established by Nees von Esenbeck (**7**) because of the presense of "Kügelchen . . . nach dem Austreten," has since been augmented on the basis that the zoöspores encysted immediately after emergence at the mouth of the sporangium in the form of a hollow sphere and later swarmed as laterally biflagellate cells, after which germination occurred. Such a mode of behavior has repeatedly been confirmed by a number of workers from 1872 on and is justly regarded as one of the main characteristics of the genus (**2**, **3**, **8**).

In the characterization of these and other genera of the Saprolegniaceae, the cycle of zoöspore swarming has been established as the basis of the generic concept. Under normal environmental conditions, the zoöspore cycles have been accepted as fairly constant, although under varying cultural influences minor changes have been reported to occur (**1**, **5**). If the amount of nutrient present is above a certain threshold, the encysted spore germinates

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. 179.

[2] The author expresses his indebtedness to Professor William H. Weston, Jr., under whose guidance and inspiration this work was carried on, for his most valuable suggestions and encouragement.

by a small tube which eventually forms a mycelium; but if the quantity of nutrient material is below this value, then the encysted zoöspore emits an active, laterally biflagellate swarmspore. There are other environmental factors, such as temperature and oxygen tension, which play an important role in determining whether the encysted spore gives rise to a hypha of germination or to an active, motile entity.

For many years, the cycles of zoöspores swarming in the various genera were considered alike in their end point—that is, they were terminated by a single swarming of secondary zoöspores. In 1919, however, Weston (**9**) reported in a non-sexual *Dictyuchus* that after the secondary, laterally biflagellate zoöspores had encysted, they might emit a second swarming stage. This repeated zoöspore emergence occurred under normal conditions, and as far as Weston was able to observe, the second set of laterally biflagellate zoöspores was exactly like the original one that had emerged from its unit within the sporangium. In 1932, also, Höhnk (**4**) reported a second emergence of laterally biflagellate zoöspores in *Saprolegnia torulosa* De Bary, and both a second and third in *Achlya racemosa* Hildebrand.

It therefore seemed of interest to determine the extent to which this repeated emergence of secondary or laterally biflagellate zoospores could be carried when environmental conditions were at an optimum for swarming. Accordingly, in this paper, there are presented observations showing that in a non-sexual, undetermined species of *Achlya* the usually described life cycle may be so modified as to include five successive swarmings before germination takes place. Since, to the writer's knowledge, such a degree of swarming has never before been reported, it appears desirable to record its occurrence in the following note.

The *Achlya* referred to in this paper was isolated from some mud gathered from a pond in the Blue Hills region of eastern Massachusetts. It was kept in culture for over eight months, and at no time displayed any evidence of sexuality, although zoösporangia were produced abundantly. Thus, it was impossible to determine the species exactly. Whether this condition was due to the fact that the species of *Achlya* was neutral or one of the strains of a heterothallic form has not been determined.

A pure culture was obtained from a single zoöspore by picking up several encysted spores in a thin, sterile pipette, streaking them rapidly along the surface of a solid nutrient medium, and about

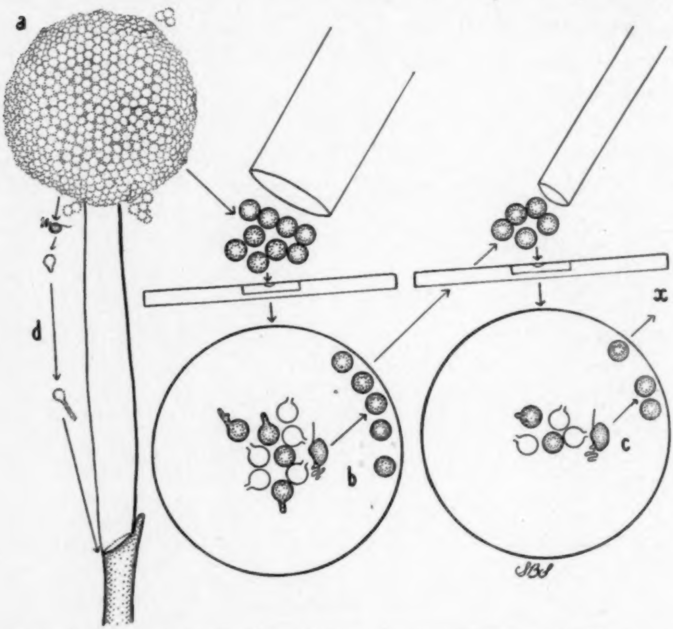

FIG. 1. Diagram illustrating quintuple emergence of the secondary zoospores in *Achlya* sp. The zoöspores emerged from the sporangium to form a hollow sphere (*a*). The encysted spores were transferred by means of a pipette to a hanging drop, where the individual spores swarmed for a period and then encysted (*b*). These quiescent spores were then transferred with another pipette to a second hanging drop, where after a while they swarmed for a time, only to encyst again (*c*). Continuing from (*x*), the repeated emergence of secondary zoöspores was carried on three additional times, making five swarming stages in all. At (*d*), the normal cycle is illustrated.

twenty-four hours later removing one of the resulting microcolonies onto another agar surface. A stock culture of the fungus was maintained on 0.3 per cent maltose-0.1 per cent peptone agar, but when zoöspores were desired, the fungus was transferred to a bit of hemp seed in glass-distilled water. The observations on the swarmings of the zoöspores were then made in a hanging drop in a depression slide.

The mycelium in its morphological characteristics and the sporangium in its development were quite similar to those in other species of *Achlya*. The zoöspores, 16 micra long and 12 micra wide, were emitted in the usual manner, forming a somewhat irregular hollow sphere of encysted zoöspores, each 13 micra in diameter, at the sporangial apex. Each spore was uninucleate and contained several tiny refractive globules. If nutrient was lacking, each of these quiescent cells emitted a laterally biflagellate zoospore, which after a period of activity again encysted. This cycle of asexual spore formation agreed with the usual accounts. In the *Achlya* under discussion, however, when conditions were optimum, four additional emergencies of secondary zoöspores were seen to occur.

This sequence of events was revealed under the following conditions: A young sporangium with some of the attached hypha was cut from a mass of mycelium which was growing on a hemp seed in sterile water. This sporangium was then transferred to a hanging drop of well-aerated water kept at a temperature of about 15° C. In this drop, after the sporangium had matured in about 2½ hours, the zoöspores emerged from the terminal papilla to form a hollow sphere at the sporangium mouth (FIG. 1, *a*).

This spore ball was then transferred to the center of another similar hanging drop by means of a micropipette. The zoöspores began to emerge from the encysted state after about thirty minutes, swam about, and finally came to rest mainly along the edge of the suspended drop (FIG. 1, *b*). These encysted zoöspores from the edge of the drop were then transferred to another hanging drop of pure, cool, sterile, well-aerated water and their positions marked in order that there might be no possibility of confusing these with the ones that emerged and encysted subsequently. After about an hour and a half, while, to be sure, some of the zoöspores germinated by sending out germ tubes, others emitted the characteristic secondary zoöspore. These in turn finally came to rest and encysted at the edge of the hanging drop (FIG. 1, *c*), whence, in the manner already described, they were transferred to the center of another similar drop. This process was repeated three more times, thus yielding a total of five successive swarmings of motile laterally biflagellate zoöspores. After the fifth emergence, the remaining

encysted zoöspores would apparently no longer emit a motile entity, for all germinated by sending out short hyphae of germination.

Five successive swarmings of the secondary zoöspore did not take place in every trial. In fact, out of six experiments, only two were characterised by this number of emergences, although every one did have some degree of repeated swarming. The zoöspores of the different swarming stages did not seem to vary in size or shape, although the amount of globular material in the zoospore seemed gradually to decrease.

The significance of this quintuple emergence of the laterally biflagellate zoöspore may be considered from various aspects. It obviously is of great survival value to the fungus. The active zoospore phase is able to continue over a much longer period of time and cover a far greater distance, thus increasing enormously its chances of finding a suitable substratum. Furthermore, this repeated swarming offers another example of "rejuvenation," in which a certain mass of protoplasm may enter into a quiescent state to emerge later as a thoroughly revived entity. If a zoöspore, on emerging from the encysted condition, is unable to find a suitable substance on which it may grow, it can pass again into a resting state, become rejuvenated, and then once more emerge as an active entity and have an opportunity of finding a favorable substratum on which it may form a germ tube and thus start a new colony. This quintuple swarming also adds to the evidence that the secondary, laterally biflagellate zoöspore is a far more efficient reproductive cell than the primary, terminally biflagellate one.

It is highly probable that the phenomenon of repeated emergence may be continued a still greater number of times if certain conditions are met. The water in which the experiments are carried on should be extremely pure, kept at a relatively low temperature (about 15° C.), and be well aerated. Since this rejuvenescence of motile zoöspores is dependent on stored energy and thus on reserve food material, other species may be found to have even more abundant storage material permitting a still greater number of emergence stages. It also appears that there may be some inherent factor present which in some way limits or controls the emergence of the zoöspores from the encysted state, since, under similar environmental conditions, the author, like Weston, was un-

able to induce the repeated emergence of zoöspores in *Thrausto-theca clavata* (De Bary) Humphrey.

In any case, the fact that in the present species of *Achyla* the writer succeeded in securing five swarming stages of secondary zoöspores, while hitherto the maximum number of emergences of motile laterally biflagellate entities was three, as reported by Höhnk in *Achlya racemosa* Hildebrand, seems to indicate that this aspect of asexual reproduction in the Saprolegniaceae is an interesting one worthy of further investigation.

SUMMARY

1. Observations on the zoöspore phase of an *Achlya* lacking sexual reproduction and therefore of an undetermined species were carried on under optimum conditions.

2. When the zoöspores are kept in cool, redistilled, well-aerated water, five successive swarmings of the secondary, laterally biflag-gelate zoöspore have been observed to occur. Such a number of emergences is two more than has ever been reported in any species of the Saprolegniaceae.

3. The exact mechanism bringing about this repeated emergence is still in doubt, although it seems to involve both environmental and hereditary factors.

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A NEW SPECIES OF TAPHRINA ON ALDER

W. WINFIELD RAY [1]

(WITH 2 FIGURES)

In a recent paper by the writer (**4**) several species of *Taphrina* occurring on various species of *Alnus* in North America were described and discussed. Received too late to be included in his paper was a new species of *Taphrina* causing leaf-curl of *Alnus rubra* Bong. The specimens, forwarded to the writer for study by Dr. Lee Bonar, were collected near Trinidad, Humboldt Co., California, March 24, 1931, by H. E. Parks.

According to the collector, the trees of Humboldt Co. are often seriously affected by the fungus. The infection is apparent on the leaves when they first appear in the spring, causing them to become greatly enlarged, often several times their natural size (FIG. 1). The leaves are curled and distorted and have a decided purple color. After the asci have matured and discharged their spores, the leaves shrivel, dry up and fall, and then a new crop of healthy leaves appears.

The discovery of a new species of *Taphrina* on *Alnus rubra* brings the total number of species on that host to three. The writer (**4**) has reported the bracts of the female catkins of this plant to be affected by *Taphrina amentorum* (Sad.) Rost. and *T. occidentalis* Ray. The two fungi affecting the female catkins, however, differ not only from one another, but also from the species occurring on the leaves. It has been noticed by the collector that *Alnus rhombifolia* Nutt. growing nearby does not become infected by the leaf-curling fungus.

The mycelium of the fungus affecting the leaves of *A. rubra* is confined strictly to the subcuticular region on the upper and lower surfaces. This mycelium constitutes the layer of ascogenous cells,

[1] The writer wishes to express his appreciation to Dr. Lee Bonar for making the material available for study, and to Miss A. E. Jenkins for the loan of the specimen mentioned in the text.

each of which eventually develops into an ascus with the absence of a basal cell. The asci are cylindrical with rounded to truncate apices, and they measure 40–55 $\mu \times$ 12–19 μ (FIG. 2*B*). The basal

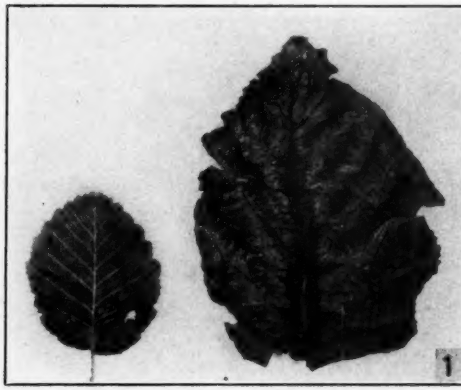

FIG. 1. Healthy leaf of *Alnus rubra* and leaf affected by *Taphrina macrophylla*, ½ nat. size.

portion of the ascus is often flat and occasionally may attain a width of 32 μ. Nearly all asci contain numerous spores, although a few were seen with eight.

Two leaf-invading species of *Taphrina* on *Alnus japonica* Sieb. and Zucc. have been reported from Japan. Both of these have asci lacking a basal cell, and in this respect they are like the species from California. The first of these, *T. japonica*, described by S. Kusano (**1**) has asci measuring 63–90 $\mu \times$ 16–25 μ, whereas the second, *T. Alni-japonicae*, described T. Nishida (**3**) has asci 60–80 $\mu \times$ 16–25 μ. Although the writer has not had an opportunity to compare these two species first hand, the host relationship and the similarity in size of the asci of the two species suggest that they may be identical. Mix (**2**) points out that these two Japanese species may be alike.

Specimens of *T. japonica* collected by S. Kusano near Tokyo, Japan, June 9, 1907 (communicated by Miss A. E. Jenkins), have been examined microscopically. The asci of this species are as described by Kusano (**1**) and are considerably larger than those

from the specimens collected in California (FIG. 2*A*). The basal portion of the asci is rounded and not widened as is often the case of the asci on the leaves of *A. rubra*. Kusano reports that *T. japonica* causes a "witches'-broom," whereas, the California species does not.

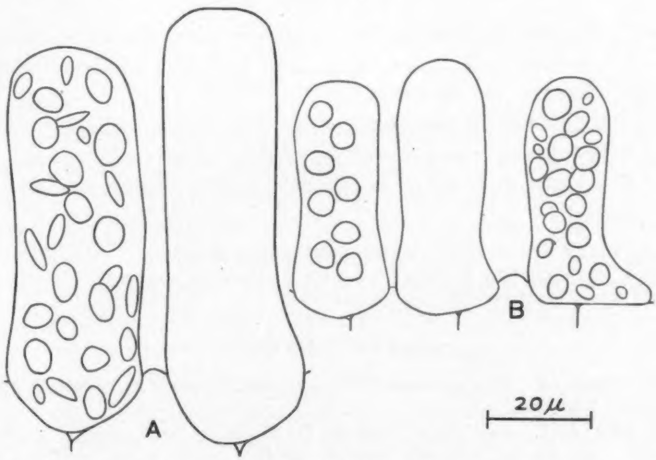

FIG. 2. *A*, asci and spores of *Taphrina japonica; B*, asci and spores of *Taphrina macrophylla*. Drawings made with the aid of a camera lucida; × 787.

On the basis of the morphology of the asci and the types of symptoms produced, the writer feels that the species affecting the leaves of *A. rubra* is distinct from *T. japonica* and has hitherto been undescribed.

Because of the large size of the leaf produced as a result of fungous invasion, the name suggested for the fungus is as follows:

Taphrina macrophylla sp. nov.

Hymenio subcuticulari; mycelio interiore carente; ascis amphigenous, cylindraceis, in apice rotundatis aut truncatis, 40–55 μ longis × 12–19 μ crassis, circa 48 × 16 μ; ad basim rotundata vel complanata, usque 32 μ crassis; cellula basalari carente; sporidiis octonis vel multis, sphaeroideis vel ellipsoideis, 2.5–5.5 μ × 2–5 μ.

Distribution: Causing leaf-curl and distortion of *Alnus rubra* in Humboldt Co., California, U. S. A.

TYPE: In the herbarium of the Department of Plant Pathology, Cornell University, No. *28831*.

ISOTYPE: In the herbarium of the University of California, H. E. Parks No. *3592*. Herbarium of W. W. Ray No. *500*.

SUMMARY

Taphrina macrophylla, a fungus causing a leaf disease of *Alnus rubra* in California, is described. The symptoms of the affected leaves likewise are described.

A comparison of this fungus with *Taphrina japonica* is made, and the differences and similarities of the two are indicated.

The host, *Alnus rubra*, is affected by three distinct species of *Taphrina*.

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TWO NEW GASTEROMYCETES

John B. Routien

(with 23 figures)

GASTERELLOPSIS SILVICOLA

During the summer of 1938 the author collected a number of soil samples near near East Lansing, Michigan, to determine whether or not *Gasterella lutophila* Zeller & Walker could be found. The results of that study may be found in a previous paper.[1] In addition to *G. lutophila,* fruiting-bodies of another fungus developed during the last of July, 1938, on one of the soil samples that came from a particular wood-lot. On October 11 samples of soil were obtained in triplicate from the same spot from which the earlier sample had been taken. Fruiting-bodies of this same new fungus were first observed on one of these collections on October 29. On a second lot of the soil fruiting-bodies did not appear until November 7, 1938.

The methods of study were the same as those employed for *Gasterella lutophila.*

Because of its similarity in many particulars to *Gasterella* the writer suggests the generic name *Gasterellopsis* for this new fungus.

The basidiocarps of *Gasterellopsis* were first visible on the surface of the soil in from 18 to 26 days after the soil was collected and prepared. The first sign of the fungus was the appearance of minute wefts of loose, white hyphae. These developed into the mature fruiting-bodies in four to six days. Most of the specimens developed on the surface of the soil, but occasionally a specimen formed just below the surface and pushed the soil particles aside as it grew.

A scarcity of specimens between the youngest and mature stages prevented discovery of the details of development of the glebal

[1] Routien, John B. Observations on *Gasterella lutophila.* Mycologia **31**: 416–418. 1939.

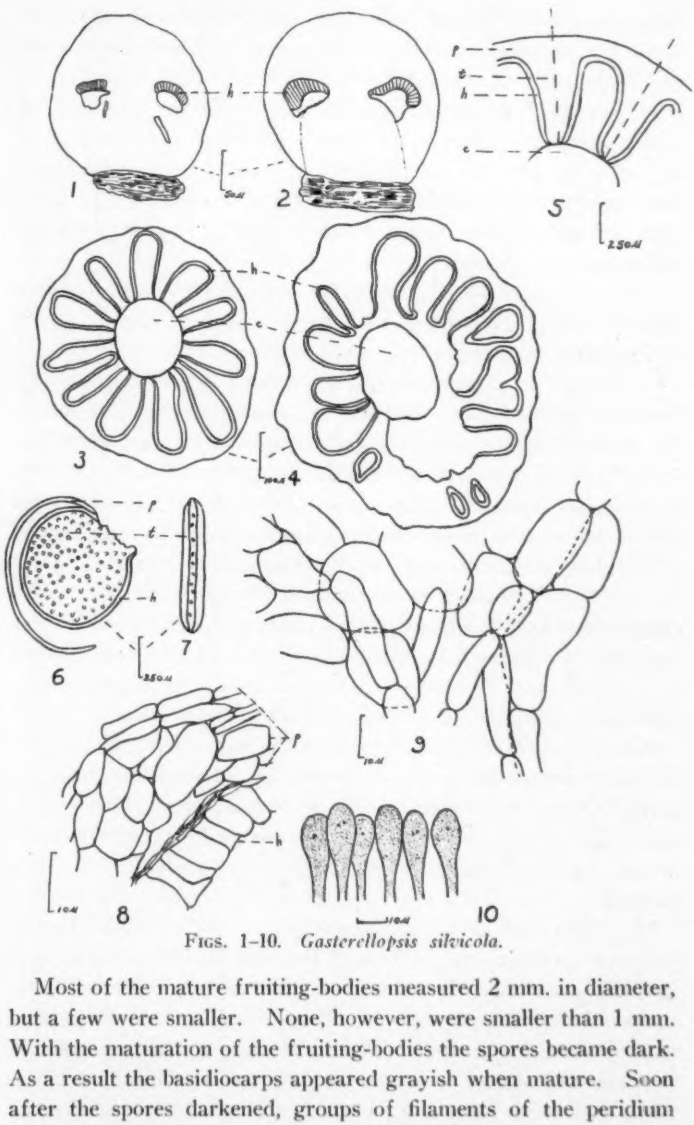
chamber. However, it seems that the cavity develops in the manner outlined below.[2]

The youngest sectioned specimens were 200 μ in diameter. In them the hymenium was just beginning as a layer of darkly-staining, downward-directed cells (FIG. 1, *h*). The cells of this layer were two-nucleate. Below this young hymenial layer there was a small circular cavity around a columella. In some young specimens (FIG. 2) it appeared as if the glebal cavity may form as a result of the tearing of tissue below the young hymenium as the fruiting-body increases in size. As the basidiocarp grows this cavity becomes enlarged.

At the same time that the basidiocarp is enlarging, tramal plates begin to develop. Apparently they originate as outgrowths of hymenium-bearing tissue from the walls of the glebal cavity. It could not be definitely established, but it seems that any particular infolding develops downward from the top, inward from the side and upward from the bottom of the basidiocarp. In the fruiting-bodies secured in the late fall all of the infoldings eventually had grown centripetally into contact with the columella, thus dividing the originally simple cavity into several distinct radially-arranged cavities (FIG. 3). In those specimens secured in the summer there were many mature fruiting-bodies in which some of the tramal plates extended from a third to a half of the distance toward the columella (FIG. 4). In some of these basidiocarps small hymenial cavities interspersed between tramal plates were also found (FIG. 4).

A peculiar feature of the fungus was discovered during observation of the living fruiting-bodies. It was found that the basidiocarp (with the exception of the columella) could easily be broken into 12–14 segments. Each segment was formed by the splitting of adjoining tramal plates (as indicated by the broken line in figure 5). Thus the sides of the peridiole-like segments were the torn or exposed tramal tissues (FIG. 6). The appearance in any segment of the edge next to the columella can best be explained by reference to the drawing of such a structure (FIG. 7).

[2] In a personal communication Dr. Leva B. Walker has informed the writer of the manner in which the basidiocarp of *Gasterella lutophila* develops. The stages of the development of *Gasterellopsis* can be interpreted in the same manner.



FIGS. 1–10. *Gasterellopsis silvicola.*

Most of the mature fruiting-bodies measured 2 mm. in diameter, but a few were smaller. None, however, were smaller than 1 mm. With the maturation of the fruiting-bodies the spores became dark. As a result the basidiocarps appeared grayish when mature. Soon after the spores darkened, groups of filaments of the peridium began to project from the surface of the basidiocarp as a result of

failure to grow while the basidiocarp was increasing in size. At this stage the peridium could very easily be removed from all of the fruiting-body except the top.

The mature fruiting-body consists of a peridium, a single glebal cavity traversed lengthwise through the center by a columella that is continuous with the fruiting-body at the top and tramal plates that project from the wall of the cavity to or almost to the columella. The hymenium covers all surfaces of the cavity except the columella.

The peridium is 18–25 μ thick and is composed of more or less inflated cells (FIGS. 8, 9) in the form of distinct hyphae.

The columella is evident in the youngest basidiocarp sectioned (FIG. 2). In a mature fruiting-body it is much taller and thicker (FIGS. 3, 4, 11, 12, 13). Although it seems to be continuous with the top of the basidiocarp, the columella is very easily pulled out from the fruiting-body because of the weakness of the flesh. This often happens when the specimens are being removed from the soil. The columella is composed of longitudinally-directed hyphae.

The tissue at the base of the fruiting-body is apparently quite weak. This is indicated by the fact that the columella is so easily removed and by the fact that the adjoining tissue becomes loosened from the base of the columella (FIGS. 11, 12, 13). The edges are then pulled away from the columella in such a manner that the basidiocarp looks very much like a diminutive mushroom.

When the fruiting-bodies were as much as six days old the cells of the peridium dissolved. This left the subhymenial layer exposed. At the same time dissolving of the tramal plates into a moist mass left the glebal cavity empty except for the spores. As a consequence of these changes, the mature basidiocarp becomes unilocular.

The hymenium consisted of paraphyses and basidia. In the youngest specimen studied most of the cells of the developing hymenium possessed two nuclei each. In the same specimen uninucleate basidia were found (FIG. 10). Nuclei in the basidia were not seen in the process of division. However, two-nucleate and four-nucleate basidia were observed (FIG. 14); the nuclei in the latter case were 1.3 μ wide. In a few basidia six nuclei were observed.

The basidia bore 2–4 basidiospores. It seemed that whenever two spores were formed, two nuclei remained in the basidium (FIG. 15). In at least some cases, when there were four spores to each basidium, four nuclei remained in the basidium (FIG. 17). All of this, of course, indicates that the four nuclei formed by the meiotic divisions may divide once more.

The basidiospores are citriform, apiculate, brownish-black and verrucose (FIG. 18). Each spore is provided with a pedical that measures $2.0 \times 1.5\ \mu$. Spores of the fruiting-bodies collected in the fall measured 14.5 (16.5)–18 $\times$ 11 (13)–14.5 μ, but the spores of the fruiting-bodies collected in the summer measured 13.5 (14.6)–16.2 $\times$ 10.8 (11.2)–12.6 μ. All of these measurements were made from permanent slides of the fungus.

It might be suggested that this fungus is a depauperate *Coprinus*. It does resemble that genus in several respects. The structure of the cells of the peridium, the origin of the circular glebal cavity around the columella and the dissolving of the trama are similar in the two genera. However, *Gasterellopsis* is unlike *Coprinus* in that in the former genus the dissolved trama does not form a liquid, the basidia are not like those of *Coprinus,* there is a splitting centripetally through the tramal plates rather than between them as is possible in *Coprinus* and the spores are not at all like those of *Coprinus.*

The fungus here described is much like *Gasterella.* The basidiocarp appears to develop in a similar manner in regard to the origin of the hymenium, and the spores are of the same type. The fungi differ, however, in several other points. The peridium of *Gasterella* is of filamentous cells, but in *Gasterellopsis* it is made up of inflated cells. The greatest differences are the presence, in *Gasterellopsis,* of tramal plates, an annular cavity and a columella as well as the opening of the basidiocarp at the base and the dissolving of the peridium and tramal plates.

In spite of these differences *Gasterella* and *Gasterellopsis* appear to the writer to be sufficiently alike to warrant their inclusion in the same family. When *Gasterella* was described,[3] it was stated that it ". . . should doubtless be referred to a new family, but

[3] Zeller, S. M. & Leva B. Walker. *Gasterella,* a new uniloculate Gasteromycete. Mycologia **27**: 572–579. 1935.

we prefer now to include it in the Protogastraceae." Since *Gasterella* seems to be sufficiently different from *Protogaster* to warrant its being placed in a new family apart from the Protogastraceae, and since there is now another plant which agrees even less with the description of the Protogastraceae [4] than does *Gasterella*, the writer makes the suggestion that *Gasterella* and *Gasterellopsis* should be placed in a new family, the Gasterellaceae.

The final disposition of such a family must wait until careful study of all possibly related forms has been made, but at present it seems that the Gasterellaceae would be placed near the Hymenogastraceae and Hydnangiaceae.

In agreement with the growing tendency of some of the recent students of the Ascomycetes as well as of the Basidiomycetes, the author believes that much greater importance should be assigned to similarities and differences in spore structure in the Gasteromycetes. It is quite possible that there have arisen parallel series of morphological development, each series being characterized by a distinct type of spore. Perhaps this will be suggested in some revised classification of the Gasteromyceteae. In that case the Protogastraceae and the Gasterellaceae (if the family is established) would be the primitive (or terminal?) families of parallel series of development in different orders in which the spore type would be of major importance.

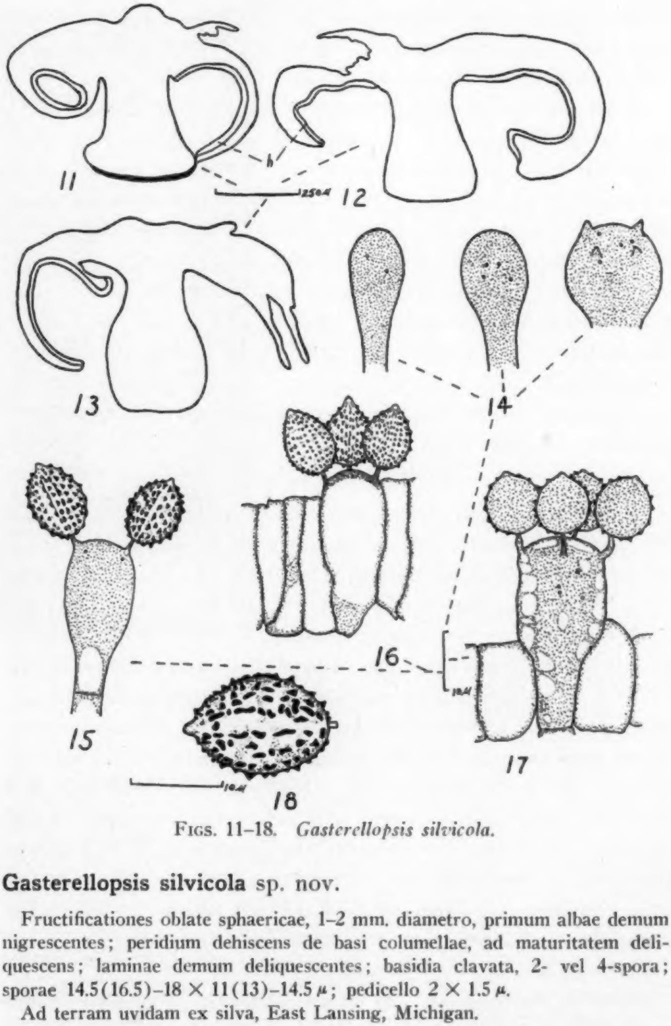
Gasterellopsis gen. nov.

Fructificationes minutae, subsphaericae; peridium ex cellulis inflatis compositum, dehiscens circumscissim ad basim; gleba uniloculata cum invaginationibus centripetalibus verticalibus paene sine usque columellam attingentibus; columella percurrens totam fructificationem ad apicem; sporae citriformes, apiculatae, verrucosae, brunnae-nigrae.

Fructifications small, subspherical; peridium of inflated cells, dehiscent in a circumscissle manner at the base; gleba uniloculate with vertical centripetal infoldings that reach usually to the columella; columella central, percurrent; spores citriform, apiculate, verrucose, brownish-black.

The type species is *Gasterellopsis silvicola*.

[4] Zeller, S. M. *Protogaster*, representing a new order of the Gasteromycetes. Ann. Missouri Bot. Gard. **21**: 231–249. *illust.* 1934.



FIGS. 11–18. *Gasterellopsis silvicola.*

Gasterellopsis silvicola sp. nov.

Fructificationes oblate sphaericae, 1–2 mm. diametro, primum albae demum nigrescentes; peridium dehiscens de basi columellae, ad maturitatem deliquescens; laminae demum deliquescentes; basidia clavata, 2- vel 4-spora; sporae 14.5(16.5)–18 × 11(13)–14.5 μ; pedicello 2 × 1.5 μ.

Ad terram uvidam ex silva, East Lansing, Michigan.

Fructifications spherical to depressed, 1–2 mm. in diameter, white, then black; peridium dehiscent at the base of the columella, dissolving at maturity; tramal plates finally dissolving; basidia clavate, 2–4 spored; spores 14.5 (16.5)–18 × 11 (13)–14.5 μ; pedicels measuring 2 × 1.5 μ.

On soil that was brought into the laboratory from the woods near East Lansing, Michigan. November, 1938. Type in herbarium of the author. Isotypes with Dr. S. M. Zeller.

Since cultures of *Gasterellopsis* would be desirable, attempts were made to germinate the spores of this fungus. The spores were plated out in potato-dextrose agar, soil-decoction malt agar, acidified potato-malt agar and acidified potato-dextrose agar. Spores also were placed in potato-dextrose broth and in solutions of 3-indoleacetic acid. This last solution was used in two concentrations: (1) 50 mg. per liter and (2) 25 mg. per liter of solution. Attempts were made to secure tissue cultures by placing bits of tissue with some of the spores in each of the media mentioned above.

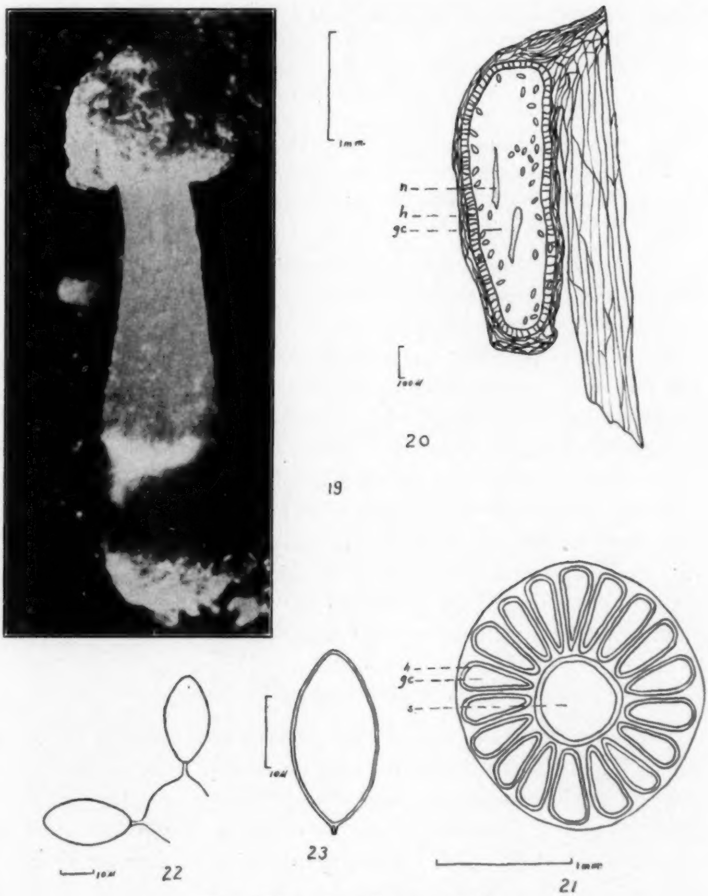
No growth of the hyphae or germination of the spores was obtained.

SECOTIUM COPRINOIDES

In the discussion of *Gasterellopsis silvicola* it was mentioned that the fungus developed only on two of the soil samples that were secured in triplicate on October 11, 1938. On the third sample there began to develop on November 12, 1938, two fruiting-bodies that greatly resembled *Gasterellopsis*. However, the fruiting-bodies became larger than that fungus and on November 15 one of them developed a stalk that elevated the fungus into the air. The other fruiting-body formed a short stalk that did not elongate. These were the only specimens that developed on this lot of soil.

The other two samples (on which only *Gasterellopsis* had formed) were again saturated with water on December 12 and the cover placed over the container. By January 12, 1939, fruiting-bodies of *Gasterellopsis* had developed on both samples. On one of the samples more specimens of the new fungus, to which the name *Secotium coprinoides* was later applied, developed: one on January 26 and one on February 3.

Some of the material was killed in formol-acetic-alcohol and prepared for sectioning and staining. Since this fungus in its earliest stages of growth looked like *Gasterellopsis*, it was impossible to secure young specimens for study of the development of the fruiting-body. All of the information regarding the fungus, therefore, is limited to the mature plant.



FIGS. 19–23. *Secotium coprinoides.*

Secotium coprinoides sp. nov.

Fructificationes albae, 4 mm. alto; peridium album, 25–35 μ crassitudine, ex cellulis filamentosis inflatis; loculi glebales circa 18; basidia 2- vel 4-spora paraphysibus intermixta; sporae ellipsoideae, leves, nigrae, 18(23.5)–30.5 × 12.6(12.75)–16 μ; pedicello 2 × 1.5 μ.

Ad terram uvidam ex silva. East Lansing, Michigan.

Fructifications (FIG. 19) 4 mm. tall, white, consisting of a stalk and a pileus-like upper portion nearly 2 mm. in diameter; this up-

per fertile region united to the stalk only near the apex of the latter (FIG. 20) and consisting of about 18 glebal chambers (FIGS. 20, 21); peridium white, of filamentous-inflated cells, 25–35 μ in thickness; hymenium at maturity consisting of basidia and paraphyses; basidia 2–4 spored (FIG. 22); spores (FIG. 23) elliptical, smooth, black, measuring (in fresh, unkilled specimens) 18 (23.5)–30.5 $\times$ 12.6 (12.75)–16 μ, each spore with a pedicel measuring 2 $\times$ 1.5 μ; two, three or four spores not uncommonly grown together and united in a group.

Developing on soil brought into the laboratory from the woods near East Lansing, Michigan, November, 1938. Type specimens in herbarium of the author.

This fungus resembles both *Gasterellopsis* and *Coprinus*. From the former it is distinguished by the stipe, the presence of a number of glebal chambers and the type of spore. From the latter it is distinguished by the presence of glebal chambers, the acrogenous position of the spores and the presence of a pedicel on each spore.

There seems to be no *Secotium* to which this new species might be closely related. *S. melanosporum* Berk. has black spores, but the fruiting-body is quite large. *S. olbium* Tul. is described as being 4–6 mm. high, but the spores are smaller than those of *S. coprinoides* and are spherical and rugulose.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the kind assistance and advice of Dr. E. A. Bessey during the progress of this study and in the interpretation of data and preparation of this paper. He also is grateful to Dr. S. M. Zeller and Dr. Leva B. Walker for their helpful suggestions. Appreciation is expressed to Professor F. C. Strong for taking the photograph of the fruiting-body of *Secotium coprinoides*.

DEPARTMENT OF BOTANY,
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EXPLANATION OF FIGURES

FIGS. 1–18 are of *Gasterellopsis silvicola*. (c) is the columella, (h) the hymenium, (p) the peridium and (t) the trama. Fig. 1, median, longitudinal section through a young fruiting-body; 2, a similar section through

an older specimen; 3, median, transverse section through a mature fruiting-body; 4, similar section showing some of the shorter tramal plates and the small, imbedded glebal chambers; 5, diagram of part of a fruiting-body indicating by the broken line how the tramal plates may be split; 6, one of the segments formed by the centripetal splitting of adjoining tramal plates; this also shows how the peridium may be removed; 7, a segment as it appears from the side next to the columella; 8, a portion of the peridium and hymenium of a mature fruiting-body in which the spores have been released from the basidia; 9, cells of the peridium; 10, basidia from a young fruiting-body showing the fusion nuclei and the nuclei formed in the first meiotic division; 11–13, mature fruiting-body; 14, two- and four-nucleate stages of meiosis; 15, two-spored basidium with two nuclei remaining in the body of the basidium; 16, three-spored basidium; 17, four-spored basidium with four nuclei in the body of the basidium; 18, basidiospore.

FIGS. 19–23 are of *Secotium coprinoides.* Fig. 19, mature fruiting-body; 20, a portion of the mature fruiting-body in a median, longitudinal section through a glebal cavity; (n) is part of a nematode; 21, median, transverse section through a mature fruiting-body showing the stipe (s) and the glebal chambers (gc); 22, two-spored basidium showing acrogenous position of the spores; 23, basidiospore.

A NEW CERCOSPORA ON LIPPIA CARDIOSTEGIA [1]

B. H. Davis [2]

(WITH 1 FIGURE)

The species of *Cercospora* described herein was found among unidentified fungous collections made by Dr. W. A. Kellerman in Guatemala in 1906 on *Lippia cardiostegia* Benth. An examination shows this to be specifically distinct from Cercosporae described on this or closely related genera. The Guatemalan *Cercospora* differs from *C. Lippiae* described by Ellis and Everhart [3] on *Lippia nodiflora* (L.) Michx. in the indefinite spots produced in its hypophyllous fruiting, dark colored conidiophores, and wide, colored conidia (FIG. 1). The following name is proposed:

Cercospora Cardiostegiae sp. nov.

Maculae indefinitae, superficie supera dilute brunneo; fungus hypophyllum, effusum, parva atra loca formans; stromata absunt vel parva, atro-fuscis; conidiophoris non-fasiculatis vel 2–12 in fasciculo, dilute ad mediocriter olivaceum brunneum, frequenter flexuosis, 1–5 septatis, interdum leniter ad septa constrictis, non-fasciculatis conidiophoris frequenter ramosis, erectis ad curvatas, 1–4 leniter ad abruptum geniculatum, apicibus rotundatis, sporarum cicatricibus parvis, 4–6.5 × 15–60 μ, plerumque 5.5–6 × 40–50 μ; conidiis dilute ad mediocriter olivaceum brunneum, rectis vel curvulis, cylindraceis ad cylindrum obclavatum, 1–7 conspicuiter septatis, plerumque 1–3 septatis, interdum ad septa constrictis, extremis abrupte rotundatis ad obconica, 4–5.6 × 20–75 μ, plerumque 4.2 × 25–40 μ.

Hab. in foliis *Lippia cardiostegia* Benth., Laguna, Depart. Amatitlan, Guatemala.

No definite leaf spots formed, upper surface light-brown; fruit-

[1] Papers from the Department of Botany, The Ohio State University, No. 422.

[2] The writer wishes to express his appreciation of the generous help of Dr. Charles Chupp in describing the species and in making available type specimens for examination. Thanks are due Dr. H. N. Moldenke of the New York Botanical Garden for his kindness in identifying the host plant.

[3] Ellis, J. B. & Everhart, B. M. *Cercospora Lippiae* E. & E. *In* Additions to *Cercospora, Gloeosporium* and *Cylindrosporium*. Jour. Myc. **3**: 20. 1887.

ing hypophyllous, effuse, forming small darkened areas; stromata absent or small, dark-brown; conidiophores non-fasciculate or 2–12 in a fascicle, pale to medium olivaceous-brown, frequently irregular

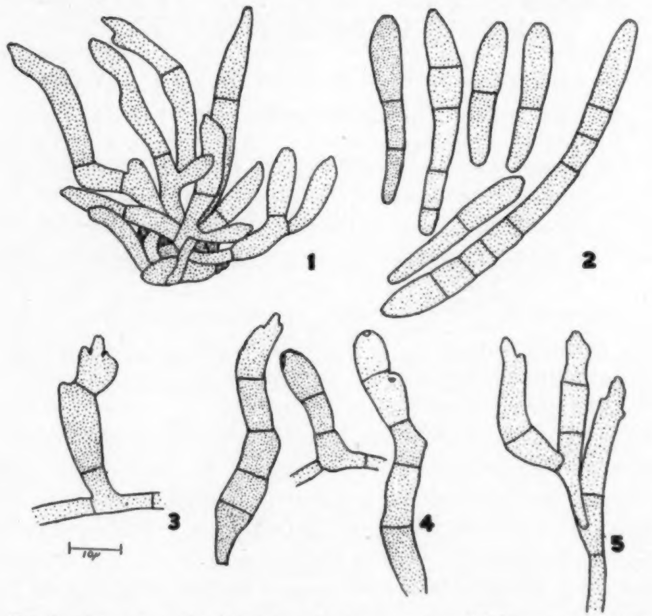

FIG. 1. *Cercospora Cardiostegiae.* 1, stroma and conidiophores; 2, conidia; 3, 4, simple conidiophores; 5, branched conidiophore.

in width, 1–5 septate, sometimes slightly constricted at the septa, non-fasciculate conidiophores frequently branched, straight to variously curved, 1–4 mildly to abruptly geniculate, tips bluntly rounded, spore scars small, 4–6.5 × 15–60 μ, usually 5.5–6 × 40–50 μ; conidia pale to medium olivaceous-brown, straight to slightly curved, cylindrical to cylindro-obclavate, 1–7 plainly septate, usually 1–3 septate, sometimes constricted at the septa, ends bluntly rounded to obconical, 4–5.6 × 20–75 μ, usually 4.2 × 25–40 μ.

On leaves of *Lippia cardiostegia* Benth., Laguna, Depart. Amatitlan, Guatemala.

Type material deposited in herbaria of Ohio State University and Cornell University.

DEPARTMENT OF BOTANY,
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ASCOMYCETES FROM THE STATE OF MINAS GERAES (BRAZIL)

CARLOS E. CHARDON, JULIAN H. MILLER AND ALBERT S. MULLER

(WITH 37 FIGURES)

The present paper on the Ascomycetes from the State of Minas Geraes, Brazil, is based almost exclusively on collections made by the junior author, who held the chair of phytopathology at the Escola Superior de Agricultura, at Vicosa, during the years 1929 to 1937.

These collections comprise about 1150 numbers, which have been kept at the herbarium of the school at Vicosa, and duplicates deposited in the herbarium of the Department of Plant Pathology at Cornell University.

The rusts of the collection have been recently studied by Dr. Frank D. Kern and Dr. W. H. Thurston of the Pennsylvania State College. The Cercosporae, constituting an important group of plant parasites, were abundantly collected and two contributions have appeared by Dr. Charles Chupp, of Cornell University and the junior author.[1] These two papers enumerate 123 species of *Cercospora*, 22 of which are new to science.

The present paper lists 114 species, 21 of which are described as new. The species of *Balansia, Claviceps, Dothichloe* and *Myriogenospora* have been determined by Dr. Wm. W. Diehl, of the United States Department of Agriculture; and the Perisporiales and Microthyriales, by Professor R. A. Toro, of the University of Puerto Rico. The writers wish to express their acknowledgments to the above collaborators.

MYRIANGIALES

1. MYRIANGIUM DURIAEI Mont. & Berk. Lond. Jour. Bot. **4**: 72. 1845.

On scale insect on *Prunus Persica*, Vicosa, *Muller 141*, Mar. 3, 1930.

[1] Archives Inst. Biol. Rio 1^3: 213–220. 1934; 3^1: 91–98. 1937.

PERISPORIALES

2. CAPNODIUM CITRI Berk. & Desm. Jour. Hort. Soc. Eng. **4**: 252. 1849.

On *Citrus* sp., Vicosa, *Muller 188*, Feb. 3, 1930.

3. CAPNODIUM THEAE Boedijn Bull. Jard. Bot. Buitg. III **11**: 223. 1931.

On *Thea sinensis*, Ouro Preto, *Muller 527*, May 1, 1933.

4. MELIOLA MATAYBAE F. L. Stevens, Ann. Myc. **26**: 226. 1928.

On *Paullinia* sp., Vicosa, *Muller 553*, May 25, 1933.

5. **Meliola Mulleri** Toro, sp. nov.

TYPE: *Cornell Univ. Explor. Brazil 632.*

Mycelii plagulae epiphyllae, orbiculares, .5–1.5 μ in diam., hyphis centrifugis, ramosis, fuscis, septatis, parietibus crassis, setis mycelicis acutis, 266 × 7 μ, dilutis brunneis, septatis, rectis; hyphopodiis capitatis alternantibus, clavatis, cellula superiore globosa, 10 μ in diam., cellula inferiore rectangulare; hyphopodiis mucronatis ampulliformibus, continuis, oppositis, 13 μ altis; perithecia globosa, in plagulis centraliter disposita, pauca lenia, leniter translucida, 120 μ in diam.; asci fugacea, sporis oblongis, utrinque obtusis, 4-septatis, 95 × 30 μ.

Mycelial colonies epiphyllous, round, .5–1.5 mm. in diam., with hyphae growing centrifugally, branched, brown, septate and thick-walled; mycelial setae acute, 266 × 7 μ, light brown throughout, septate, straight; capitate hyphopodia alternate, pear-shaped, upper cell round, 10 μ in diam., lower cell rectangular, 7 μ wide, forming an acute angle with the mycelium; mucronate hyphopodia bottle shaped, one-celled, opposite, 13 μ high; perithecia in center of spot, few, smooth, slightly translucent, 120 μ in diam., with evanescent asci; spores 4-septate, oblong, end cells rounded, 95 × 30μ.

Beelian formula : – 3111–63: 21.

On *Cardiospermum grandiflorum*, Ana Florencia, *Muller 632*, June 21, 1933.

6. MELIOLA PANICI Earle, Muhlenbergia **1**: 12. 1901.

On *Erianthus angustifolium*, Vicosa, *Muller 579*, June 3, 1933.

7. MELIOLA TERAMNI Sydow H. & P. Ann. Myc. **15**: 193. 1917.

On *Teramnus uncinatus*, Vicosa, *Muller 269*, May 2, 1931.

8. MELIOLA THOUINIAE Earle, Bull. N. Y. Bot. Gard. **3**: 308. 1905.

On *Serjania* sp., Vicosa, *Muller 552*, May 25, 1933.

9. PARODIOPSIS BRACHYSTEGIAE (P. Henn.) Arn. Annal. des Epiphyties **7**: 68–70, 1924.
On *Copaiba Longedorffii,* Vicosa, *Muller 723,* Feb. 15, 1934.

HYPOCREALES

10. BALANSIA AMBIENS Moll. Phycom. und Ascomycet. 303. 1901.
On *Leersia grandiflora,* Vicosa, *Muller 415,* Mar. 22, 1933.

11. CLAVICEPS PASPALI Stev. & Hall, Bot. Gaz. **50**: 462. 1910.
On *Paspalum intermedium,* Uberaba, *Muller 1091,* May 19, 1936.
On *Paspalum mandiocanum,* Vicosa, *Muller 434,* Mar. 30, 1933.
On *Paspalum pilosum,* Curvello, *Muller 1012,* Mar. 1, 1936.
On *Paspalum plicatulum,* Curvello, *Muller 1013,* Mar. 1, 1936.
On *Paspalum plicatulum* var. *cinereum,* Uberlandia, *Muller 1015,* Mar. 14, 1930.

12. DOTHICHLOE ATRAMENTOSA (Berk. & Curt.) Atk. Jour. Myc. **11**: 260. 1905.
On *Andropogon bicornis,* Vicosa, *Muller 19,* Nov. 10, 1929.
On *Andropogon leptostachyus,* Vicosa, *Muller 473,* Apr. 18, 1933.
On *Sporobolus Poiretii,* San Geraldo, *Muller 1137,* Nov. 17, 1929.

13. **Dothichloe discoidea** (P. Henn.) Diehl, comb. nov.
Balansia discoidea P. Henn. Hedwigia Beibl. **39**: 77. 1900.
On *Ichnanthus candidus,* Vicosa, *Muller 475,* Apr. 18, 1933.
On *Oplismenus hirtellus,* Vicosa, *Muller 641,* Apr. 18, 1933.
On *Panicum* sp., Metallurgica, *Muller 1136,* Dec. 23, 1929.

14. DOTHICHLOE LIMITATA Diehl, Jour. Agr. Res. **58**: 948. 1939.
On *Sporobolus indicus,* Ana Florencia, *Muller 768,* Apr. 1, 1934, and Sylvestre, *Muller 37,* Dec. 1, 1929.

15. DOTHICHLOE NIGRICANS (Speg.) Chardon, Mycologia **19**: 296. 1927.
Epichloe nigricans Speg. Anal. Soc. Ci. Argent. **19**: 47. 1885.

On *Panicum affolyroides*, Uberlandia, *Muller 1064*, May 18, 1936.

On *Panicum Schiffneri*, Vicosa, *Muller 490*, Apr. 22, 1933.

16. DOTHICHLOE SUBNODOSA Chardon, Mycologia **13**: 287. 1921.
On *Panicum pilosum* Vicosa, *Muller 411*, Mar. 22, 1933.

17. GIBBERELLA PULICARIS (Fries) Sacc. Michelia **1**: 43. 1879.
Sphaeria pulicaris Fries, Syst. Myc. **2**: 417. 1823.
On *Coffea arabica*, Vicosa, *Muller 706*, Jan. 3, 1934.
On dead sticks, Ponta Nova, *Muller 261*, Apr. 4, 1931.

18. GIBBERELLA SAUBINETII (Mont.) Sacc. Michelia **1**: 513. 1879.
Sphaeria Saubinetii Mont. Crypt. Alger. **1**: 476. 1846.
On *Oryza sativa*, Vicosa, *Muller 946*, June 22, 1935.
On *Paspalum intermedium*, Vicosa, *Muller 945*, June 22, 1935.
On *Triticum aestivum*, Vicosa, *Muller 148*, Feb. 20, 1930.
On *Zea Mays*, Vicosa, *Muller 311*, Feb. 25, 1932.

19. LINEARISTROMA LINEARE (Rehm.) Höhnel Sitz.-ber. Akad. Wien **119**: 939. 1910.
On *Axonopus* sp., Sao Miquel, Vicosa, *Muller 1135*, Nov. 15, 1929.

20. MYRIOGENOSPORA ACICULISPORA Viz. Bol. Agr. Sao Paulo **27**: 60–69. 1926.
On *Saccharum officinarum*, Vicosa, *C. A. Drummond 1270*, June 8, 1938.

21. MYRIOGENOSPORA PASPALI Atk. Bull. Torrey Club **21**: 225. 1894.
On *Axonopus obtusifolius*, Vicosa, *Muller 77*, Dec. 20, 1929.
On *Imperata brasiliensis*, Vicosa, *Muller 28*, Nov. 20, 1929.
On *Papsalum conjugatum*, Vicosa, *Muller 488*, Apr. 22, 1933.

22. NECTRIA CINNABARINA Tode ex Fries, Summa Veg. Scand. 388. 1849.
On *Morus alba*, Vicosa, *Muller 1032*, Jan. 15, 1936.

23. NECTRIA CONIGENA Ellis & Ev. Bull. Torrey Club **10**: 77. 1883.
On *Crotalaria juncea*, Vicosa, *Muller 454*, Apr. 12, 1933, and *Muller 973*, June 15, 1935.

24. NECTRIA SANGUINEA Fries, Summa Veg. Scand. 388. 1849.
On *Citrus* sp., *Muller 1140*, Lavras, Dec. 4, 1936.
On bark, Ponte Nova, *Muller 258*, Apr. 4, 1931.

25. PLEONECTRIA PSEUDOTRICHIA (Schw.) Wollen. Angew. Bot. **8**: 195, 208. 1926.
Sphaeria pseudotrichia Schw. Berk. & Curt. Jour. Acad. Phila. II. **2**: 289. 1853.
Megalonectria pseudotrichia Speg. Anal. Soc. Ci. Argent. **12**: 217. 1881.
On dead wood, Vicosa, *Muller 887*, Mar. 20, 1935.

26. PODONECTRIA COCCICOLA (Ellis & Ev.) Petch, Trans. Brit. Myc. Soc. **7**: 146. 1921.
On *Citrus* sp., Vicosa, *Muller 47*, Oct. 6, 1929, and Muller 53, Oct. 13, 1929.

27. SPHAEROSTILBE FLAMMEA (Berk. & Rav.) Tul. Fung. Carp. **1**: 130. 1861.
On *Ficus Carica,* Vicosa, *Muller 136*, Oct. 12, 1929.

SPHAERIALES

28. APIOSPORA APIOSPORA (Dur. & Mont.) Underw. & Earle, Bull. Ala. Exp. Sta. **80**: 186. 1897.
On bamboo cane, Vicosa, *Muller 397*, Jan. 28, 1933.

29. CERATOSTOMELLA FIMBRIATA (Ellis & Hals.) Elliott, Phytopathology **13**: 56. 1923.
On *Crotalaria juncea*, Vicosa, *Muller 1096*, Apr. 12, 1936.

30. DIATRYPE RIOGRANDENSIS Rehm, Ann. Myc. **9**: 368. 1911.
On wood, Ponte Nova, *Muller 256*, Apr. 4, 1931.
On wood, Vicosa, *Muller 496 and 500*, Apr. 23, 1933.

31. DIAPORTHE CITRI Wolf, Jour. Agr. Res. **33**: 625. 1926.
On *Citrus aurantifolia,* Vicosa, *Muller 842*, Jul. 28, 1934.

32. EUTYPELLA FRAXINICOLA (Cooke & Peck) Sacc. Syll. Fung. **1**: 154. 1882.
On dead branches, Vicosa, *Muller 495*, Apr. 23, 1933.
On dead wood, *Drummond 1117*, Feb. 15, 1936.

This is a very common species with spores 6–9 × 2 μ, and slightly protruding coarsely sulcate ostiola. It occurs on almost any kind of deciduous wood and so has been listed under many names. The correct specific name awaits a revision of the genus.

33. EUTYPELLA STELLULATA (Fries) Sacc. Syll. Fung. **1**: 149. 1882.

On *Montanua grandiflora*, Vicosa, *Muller 766*, Mar. 27, 1934.

This species differs from the above only in the possession of larger ascospores. It also is very common in most countries.

34. FRACCHIAEA HETEROGENA Sacc. Atti Soc. Ven.-Trent. **2**: 163. 1873.

On *Oncoba spinosa*, Vicosa, *Muller 391*, Oct. 12, 1933.

On dead twigs, Vicosa, *Muller 708*, Feb. 10, 1934.

35. **Guignardia atropurpurea** Chardon, sp. nov. (FIG. 26, 27, 28). TYPE: *Cornell Univ. Explor. Brazil 443.*

Maculae amphigenae, suborbiculare, 1–5 mm. in diam., numerosis, minutis, violaceis, peritheceis compositae, sine folii discoloratione; perithecia minuta, globosa, .3–.4 μ in diam., brunnea vel violacea, in mesophyllo immersa, apicis supra superficiem folii elevata, pariete brunneo pseudoparenchymato; asci clavati, 8-sporis, fasciculati, 65–75 × 13–18 μ, sporis inordinatis, continuis, hyalinis, oblongo-ellipsoideis, levibus, 17–21 × 7–8 μ, contextu granulato; sine paraphysibus.

Spots amphigenous, large, approximately circular, 1–5 mm. in diam., filled with numerous minute violet perithecia, but without leaf discoloration; perithecia small, .3–.4 μ in diam., brown to violet, ostiola papillate and elevated above leaf surface; wall brown, pseudoparenchymatous; asci clavate, 8-spored, in fascicle, 65–75 × 13–18 μ; spores inordinate in ascus; spores 1-celled, hyaline, long-elliptical, smooth, 17–21 × 7–8 μ, context granular; paraphyses absent.

The genus *Guignardia* is used here in the sense of *Guignardia Bidwellii* (Ellis) Viala & Rav.; that is, with the concept of an uniloculate stroma with neither paraphyses nor paraphysoids and differing from *Mycosphaerella* only in the possession of one-celled ascospores.

On *Miconia* sp., Vicosa, *Muller 443*, Jan. 4, 1933.

36. **Guignardia punctiformis** Chardon, sp. nov. (FIG. 29, 30, 31).
TYPE: *Cornell Univ. Explor. Brazil 852.*

Maculae amphigenae, fere orbiculare, brunneae, 4–6 mm. in diam.; perithecia in epiphyllo, pauciora in hyphyllo, aequaliter dispersa, solitaria, in mesophyllo, subglobosa, 145–180 × 108–160 μ; ostiola obtusa; asci fasciculati, clavati, octo-spori, 72–90 × 30–34 μ, breviter stipitati, apicibus .3–.4 μ crassis; sporae stipatae, hyalinae, continuae, ellipsoideae vel subpyriformae, 20–23 × 10–13 μ, leve, contextu granulato, sine paraphysibus.

Spots amphigenous, approximately circular, brownish, 4–6 mm. in diam.; perithecia conspicuous as black dots above but much less prominent below, equally dispersed, solitary, sunken in the mesophyll, subglobose, 145–180 × 108–160μ, wall membrane about 35–40 μ, thick, ostiola obtuse-papillate; asci fasciculate, clavate, 8-spored, 72–90 × 30–34 μ, short stalked, apices .3–.4 μ thick; spores crowded, hyaline, continuous, ellipsoidal to sub-pyriform, 20–23 × 10–14 μ, smooth, contents granular; without paraphyses.

On *Miconia* sp., Vicosa, *Muller 852*, Oct. 26, 1934.

37. HEPTAMERIA OBESA (Dur. & Mont.) Sacc. Syll. Fung. **2**: 88. 1883.

On dead twigs, Vicosa, *Muller 392*, Jan. 12, 1933.

38. HUMBOLDTINA BONPLANDI Chardon & Toro, Univ. Puerto Rico Monog. Biol. B. **2**: 183. 1934.

On dead wood, Vicosa, *Muller 389*, Jan. 22, 1933.

39. LEPTOSPHAERIA SACCHARI Breda de Haan, Med. Proefst. Suik., West-Java **1892**: 25.

On *Saccharum officinarum*, Vicosa, *Muller 301*, Feb. 20, 1932.

40. NEOPECKIA RHODOSTICTA (Berk. & Br.) Sacc. Syll. Fung. **11**: 317. 1892.

On dead bark, Ponte Nova, *Muller 257*, Apr. 4, 1931.

41. OPHIOBOLUS CARICETI (Berk. & Br.) Sacc. Syll. Fung. **2**: 349. 1883.

On *Oryza sativa,* Vicosa, *Muller 408*, Mar. 20, 1933.

On *Oryza sativa*, Sete Lagoos, *Muller 953*, Jul. 12, 1935.

42. **Ophiodothella Bignoniacearum** Chardon, sp. nov.
TYPE: *Cornell Univ. Explor. Brazil 453.*

Maculae amphigenae, conspicuae, grandes, orbicularae, brunneae, 4–10 mm. in diam.; perithecia numerosa, atro-punctiformia, amphigena, solitaria dis-

posita vel aggregata, leniter depresso-globosa, 220–300 × 180–216 μ, superne clypeis et membrana prosenchymata circumdata, 6–10 μ crassa, ostiolis brevibus papilliformis, periphysibus; asci cylindracei, breviter stipitati, 8-spori, 72–85 × 8–9 μ, sporis hyalinis, filiformis, continuis, stipatis, in asco; paraphyses parce evolutae, fibrosae.

Spots amphigenous, conspicuous, large, consisting of brown circular areas, 4–10 mm. in diam., provided with numerous black punctiform perithecia, visible on both surfaces of leaf; perithecia single, globose to flattened, 220–300 × 180–216 μ, with clypeus above and surrounded by prosenchymatous wall, 6–10 μ, thick, ostiole short-papillate, with periphyses; asci cylindric, 8-spored, short stalked, 72–85 × 8–9 μ; spores filiform, hyaline, 1-celled, tightly pressed together in ascus; paraphyses present but inconspicuous with age.

This genus has all of the characters of *Linospora* Fuckel with the exception of the beak. *Ceuthocarpon* Karst. is not a synonym of *Ophiodothella*, but is synonymous with *Linospora* as its type, *C. populinum* (Pers.) Karst., also possesses beaks on the perithecia.

On Bignoniaceae, Vicosa, *Muller 453*, Apr. 12, '33, and *556*, May 25, 1933.

43. OPHIODOTHELLA INGAE (P. Henn.) Theissen & Sydow, Ann. Myc. **13**: 614. 1915.

Vialaea Ingae Rehm, Hedwigia **40**: 120. 1901.

Phyllachora Ingae P. Henn. Hedwigia **48**: 8. 1908.

Scolecodothopsis Ingae Stev. Ill. Biol. Mon. **8**[3]: 183. 1923.

Diatractium Ingae H. & P. Sydow, Ann. Myc. **18**: 183. 1920.

On *Inga* sp., Vicosa, *Muller 538,* May 20, 1933, and 698, Feb. 4, 1934.

44. PARODIELLA PERISPORIOIDES (Berk. & Curt.) Speg. Anal. Soc. Ci. Argent. **9**: 178. 1880.

On *Crotalaria* sp. Sylvestre, *Muller 32*, Dec. 1, 1929.

On *Crotalaria* sp., Vicosa, *Muller 21*, Nov. 14, 1929.

On *Indigofera suffructicosa*, Vicosa, *Muller 920*, May 22, 1935.

This fungus has been placed in the Perisporiaceae, but as its perithecium is in reality an uniloculate stroma with paraphysoids, it belongs along with *Apiosporina*, *Neopeckia*, *Herpotrichia*, etc.

45. PSEUDOPLEA BRIOSIANA (Poll.) Höhnel, Ann. Myc. **16**: 163. 1918.

On *Medicago sativa* L., Machado, *Muller 754*, Jan. 20, 1934.

46. PSEUDOTHIS SUBCOCCODES (Speg.) Theissen, Ann. Myc. **16**: 182. 1918.

This fungus is identical with *Toro 359*, from Salgar, Colombia. The conspicuous, dirty brown pustule-like protuberances on the leaves contain numerous perithecia, with asci having brown, 2-celled spores, $9\text{–}10.5 \times 5\text{–}6\ \mu$. The cells are unlike. Conidia are also found in small pockets at the borders of the fructifications. They are unicellular, brown, more or less globose, $6\text{–}7\ \mu$ in diameter. See figure 14, Jour. Dept. Agr. Puerto Rico **14**: 270. 1930.

On *Dalbergia miscolobium*, Lagoa Santa, *Muller 961*, July 16, 1935.

On *Machaerium oblongifolium*, Cajury, *Muller 292*, Oct. 12, 1931, and Vicosa, *Muller 682*, Feb. 4, 1934.

On *Machaerium* sp., Cajury, *Muller 295*, Oct. 12, 1931.

47. SPHAERULINA ORYZAE Miyake, Bull. Coll. Agr. Tokyo Imp. Univ. **8**: 245. 1910.

On *Oryza sativa*, Vicosa, *Muller 409*, Mar. 20, 1933.

48. VALSA LEUCOSTOMA Pers. ex Fries, Summa Veg. Scand. 411. 1849.

On *Prunus Persica*, Vicosa, *Muller 860*, Nov. 2, 1934.

On *Prunus Persica* var. *nucipersica* Vicosa, *Muller 705*, Feb. 10, 1934.

XYLARIACEAE

49. CAMILLEA MACROMPHALA (Mont.) Cooke, Grevillea **12**: 3. 1883.

On dead trunk, Vicosa, *Muller 820*, Jul. 2, 1934.

50. CAMILLEA SAGRAEANA (Mont.) Berk. & Curt. Jour. Acad. Nat. Sci. Phila. II. **2**: 285. 1853.

On dead trunk, Vicosa, *Muller 18*, Nov. 8, 1929.

51. CAMILLEA TURBINATA (Berk.) Speg. Fungi Argent. Pug. **IV**., n. 134. 1882.

On dead trunk. Araponga, Canaan, *Muller 340*, Apr. 29, 1932.

52. HYPOXYLON ANTHRACODES (Fries) Mont. Ann. Sci. Nat. II. **13**: 359. 1840.

On dead bark, Ponte Nova, *Muller 260*, Apr. 4, 1931.

53. **Hypoxylon applanatum** (Theissen) J. H. Miller, comb. nov.

Nummularia commixta Rehm v. *applanata* Theissen, Ann. Myc. **6**: 350. 1908.

The stroma is plane to convex depending on the shape of the wood, of indefinite dimensions, discrete, pulverulent, later shining black; surface is smooth with slightly raised hemispheric ostiola, widely punctate with age; ascospores fusoid-elliptical, 25–32 × 6–8 μ.

This fungus has no connection with *Nummularia commixta* Rehm. The latter should bear an earlier name, *Nummularia scriblita* (Mont.) Cooke. It is a typical *Nummularia* with a circular stroma with abrupt walls, and perithecia deeply sunken in the stroma, with ostiola in cavities with wide pores and slightly raised borders. The American form, which it resembles, *Hypoxylon mediterraneum* (DeNot.) J. H. Miller, has more prominent strongly papillate ostiola and smaller spores.

On dead wood, Vicosa, *Muller 374*, Oct. 15, 1934.

54. HYPOXYLON CULMORUM Cooke, Grevillea **7**: 51. 1878.

On *Merostachys speciosa*, Vicosa, *Muller 817*, June 9, 1934.

55. **Hypoxylon folicola** J. H. Miller, sp. nov.

TYPE: *Cornell Univ. Explor. Brazil 10.*

Stromata ad superficem folii, dispersa vel aggregata, irregulariter pulvinata v. globosa, atro-brunnea, v. atria, verrucosa v. tuberculates, 1–2 mm. in diam., et .5–1 mm. alta, carbonacea; perithecia .5 mm. in diam., 2–6 in quoque stromate; ostiola colla brevi papillata v. indistincta; asci cylindracei breviter pedicellati, 8-spori, 90–120 × 12–14 μ, par. spor. 70–95 μ longi; sporis oblongis v. navicularibus diluto-brunneis v. atro-brunneis, 16–20 × 7–9 μ; paraphyses praesentes.

Stromata superficial on the leaf surface, dispersed to aggregated, pulvinate to globose, dark brown varying to black in age, surface verrucose to tubercular, 1–2 mm. in diam., and .5–1 mm. high, carbonous; perithecia .5 mm. in diam., 2–6 in each stroma; ostiola necks small, papillate to indistinct; asci cylindrical, briefly stalked, 8-spored, 90–120 × 12–14 μ, spore part 70–95 μ long; spores oblong to navicular, dilute brown to dark brown, 16–20 × 7–9 μ; paraphyses present.

H. verrucosum Theissen, *H. megalosporum* Speg., and *H. umbrino-velatum* Berk. & Curt. all are similar in appearance, but differ in possession of much larger ascospores.

The nearest approach is one in Kew Botanical Garden labelled *H. Kurzianum* Curr. on palm and bamboo leaves. However, this is an undescribed name.

On *Palmae*, Vicosa, *Muller 10*, Oct. 17, 1929.

56. HYPOXYLON TRUNCATUM (Schw. ex Fries) J. H. Miller, Trans. Brit. Myc. Soc. **17**: 130. 1932.

Sphaeria truncata Schw. Syn. Car. 174. 1822.

Sphaeria truncata Schw. ex Fries, Syst. Myc. **2**: 422. 1823.

Sphaeria annulata Schw. Jour. Acad. Nat. Sci. Phila. **5**: 11. 1825.

Sphaeria annulata v. *depressa* Fries, Elench. Fung. **2**: 64. 1828.

Sphaeria marginata Schw. Trans. Am. Phil. Soc. II. **4**: 190. 1832.

Sphaeria truncatula Schw. Trans. Am. Phil. Soc. II. **4**: 210. 1832.

Hypoxylon annulatum Mont. In C. Gay, Hist. Chile Bot. **7**: 445. 1850. (Excl. spec.)

Hypoxylon marginatum Berk. Outl. Brit. Fungol. 387. 1860.

Rosellinia nitens Ces. Note Bot. **13**: 1872.

Hypoxylon chalybeum Berk. & Br. Jour. Linn. Soc. **14**: 121. 1875.

Hypoxylon glomiforme Berk. & Curt. Grevillea **4**: 49. 1875.

Hypoxylon Murrayi Berk. & Curt. Grevillea **4**: 49. 1875.

The name *truncatum* is the oldest epithet and has the added validity of occurring in Systema Mycologicum. Schweinitz (Trans. Am. Phil. Soc. II. **4**: 210) substituted the name *truncatula* for his earlier name of *truncata*, and (l. c. p. 190) substituted the name *marginatum* for his previous *annulata*. Types representing these names labelled by Schweinitz are in Kew Gardens, and all of them are phases of the same species.

This fungus occurs in the tropics and semitropics all over the world. There is a greenish conidial layer, followed by a very hard, black, carbonous stroma, inclosing perithecia with an annulate depression around each ostiolum. The ascospores are about $9 \times 3\ \mu$.

The other species, *H. stygium* (Lév.) Sacc., has a similar ap-

pearance, but differs in smaller ascospores and in beginning as a reddish layer instead of green.

On dead bark, Vicosa, *Muller 24*, Nov. 15, 1929; *Muller 375*, Oct. 16, 1932; *Muller 498*, Apr. 23, 1933; *Muller 707*, Feb. 10, 1934.

57. KRETZSCHMARIA CETRARIOIDES (W. & Curr.) Sacc. Syll. Fung. **9**: 567. 1891.

On dead stump, Vicosa, *Muller 31*, Nov. 24, 1929.

58. **Penzigia enteroleuca** (Speg.) J. H. Miller, comb. nov.

Hypoxylon enteroleucum Speg. Fung. Argent. 264. 1898.

This fungus lies within the concept of *Penzigia* Sacc. in possessing white, woody internal tissue as in most *Xylaria* species, and in the pulvinate form of *Hypoxylon*, but with a basal constriction resembling a stipe. It differs from *Penzigia Berteri* (Mont.) Mill. in the absence of the coarse scales, and in being cupulate-depressed rather than convex. The spores in both species are about the same, 12–14 $\times$ 5–6 μ.

On dead wood, Vicosa, *Muller 42*, Sept. 20, 1929.

59. ROSELLINIA BRESADOLAE Theissen var. MINOR Theissen, Ann. Myc. **6**: 351. 1908.

On dead branches, Vicosa, *Muller 494*, Apr. 23, 1933.

60. ROSELLINIA SUBVERRUCULOSA Rehm, Ann. Myc. **5**: 526. 1907.

On *Bambusa* sp., Vicosa, *Muller 398*, Jan. 28, 1933; and *Muller 424*, Mar. 24, 1933.

On *Chusquea* sp., Vicosa, *Drummond 1118*, Feb. 15, 1936.

61. THAMNOMYCES CHAMISSONI Ehr. Horae, Physic. Berol. Bonn. 79. 1820.

Xylaria Chamissonis Sacc. Syll. Fung. **1**: 345. 1882.

This genus differs from *Xylaria* in the filiform-branching character of the stroma, and in the possession of single perithecia at the ends of the branches. The species is well illustrated by Moller, Phycomyceten und Ascomyceten, table 10, fig. 3, 1901.

On bark, Rio Casia, *Drummond 1120*, July 12, 1936.

62. XYLARIA ALLANTOIDEA Berk. Jour. Linn. Soc. **10**: 380. 1869.

This species differs from the common *Xylaria cubensis* Mont. in possessing more of a copper color rather than brown, and in slightly larger spores; 13–16 × 5–7 μ in the former and 10 × 4 μ in the latter.

On dead wood, Ponte Nova, *Muller 259*, Apr. 4, 1931.

63. XYLARIA ANISOPLEURA Mont. Syll. Crypt. 204. 1856.

On dead bark, Araponga, *Drummond 902*, Apr. 19, 1935.

64. **Xylaria coccinea** J. H. Miller, sp. nov.

TYPE: *Cornell Univ. Explor. Brazil 900.*

Stroma solitaria, erecta, recta, compressa, clavata, involuta, 10–20 × 5–8 mm., apicibus obtusis, basi in stipitem attenuata, crusta coccinea, ostiolis atris protrudentibus, intus pallida, lignosa; stipite cylindrico, 4–5 mm. longo, 2–3 mm. crasso, atro, glabra; perithecia immersa, atria, globosa, .5 mm. in diam.; asci cylindracei, stipitati, pars, spor. 80–110 × 7–10 μ, stipite 30–40 μ longis; octospori; sporis monostichis, cymbiformibus, continuis, fuscis, 18–24 × 6–8 μ; paraphyses filiformes.

Fertile stroma compressed clavate, 10–20 × 5–8 mm., pellicle smooth, bright red, with black protruding ostiola, involute in age, interior pallid, woody; stipe 4–5 mm. in length and 2–3 mm. in diameter, smooth, black; perithecia immersed, globose, .5 mm. in diam.; asci cylindrical, stalked, spore part 80–110 × 7–10 μ, with a stalk 30–40 μ long, 8-spored; spores uniseriate, elliptic-oblong, plano-convex, dark brown, 18–24 × 6–8 μ; paraphyses numerous and filiform.

This is one of the smooth forms with a bright colored pellicle, and so falls in a group with *X. tabacina,* and *X. enterogena* Mont. The spores in *X. coccinea* are smaller than in the former, but about the same size as those of the latter. It differs from *X. enterogena* in having the bright red color instead of the pale yellow of the latter.

On dead wood, Araponga, *Drummond 900*, Apr. 14, 1935.

65. XYLARIA GRAMMICA Mont. Syll. Crypt. 202. 1856.

On dead wood, Vicosa, *Muller 972*, Aug. 15, 1935.

66. XYLARIA SCRUPOSA (Fries) Berk. Jour. Linn. Soc. Bot. **10**: 382. 1869.

On dead wood, Vicosa, *Muller 971*, Aug. 3, 1935.

On dead wood, Vicosa, *Drummond 1034*, Feb. 15, 1936.
On dead wood, Rio Casca, *Drummond 1122*, July 15, 1936.

67. XYLARIA TABACINA (Kickx) Berk. Hook. Jour. Bot. Kew Gar Misc. **6**: 225. 1854.

On leaf mold, Araponga, *Muller 901*, Apr. 19, 1935.

DOTHIDEALES

68. BAGNISIOPSIS TIJUCENSIS Theissen & Sydow, Ann. Myc. **13**: 291. 1915.

On *Miconia* sp., Vicosa, *Muller 508*, Apr. 29, 1933.

69. COCCOSTROMA MACHAERII (P. Henn.) Theissen & Sydow, Ann. Myc. **12**: 269. 1914.

Phyllachora Machaerii P. Henn. Engl. Bot. Jahrb. **17**: 524. 1893.

On *Machaerium oblongifolium*, Vicosa, *Muller 179*, May 29, 1930, and *Muller 130*, Feb. 19, 1930.

70. DOTHIDELLA TINCTORIA (Tul.) Sacc. Syll. Fung. **2**: 627. 1883.

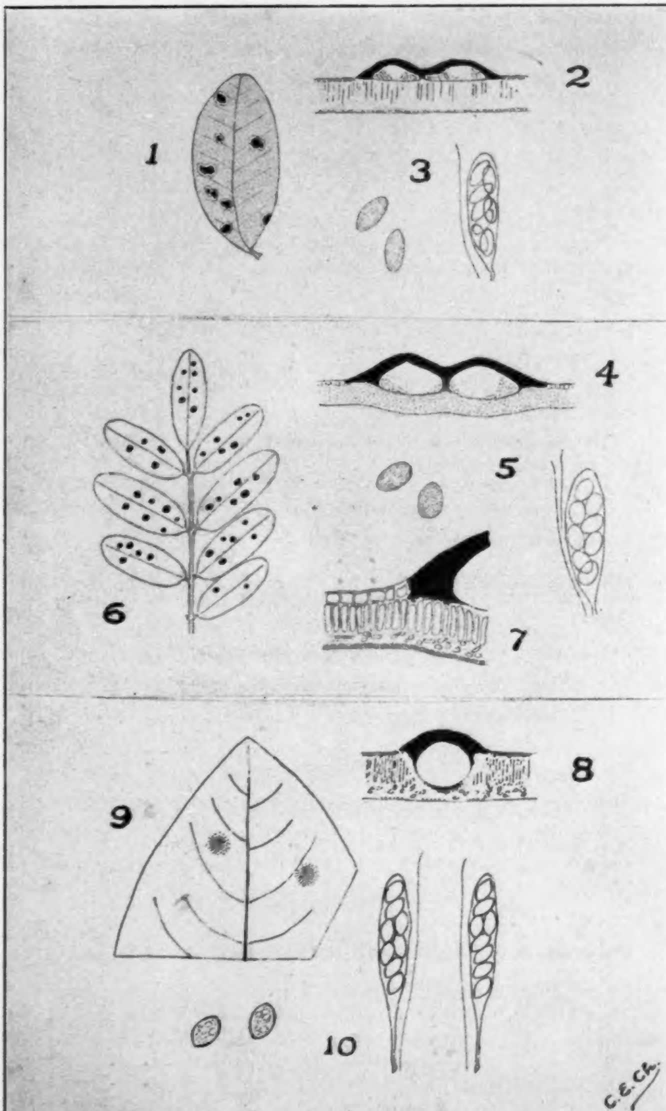
A common species on Compositae in the subtropical and temperate zones of the Andes of Colombia, Equador, and Venezuela. This is apparently the first record of the species from Brazil. The spores compare well with Colombian material. They are hyaline, 2-celled, long-elliptical, 25–29.5 × 9–11.5 μ.

On *Baccharis* sp., Cajury, *Muller 291*, Oct. 12, 1934.
On *Eupatorium* sp., Vicosa, *Muller 999*, Nov. 2, 1935.

PHYLLACHORACEAE

71. **Catacauma copaiferiicola** Chardon, sp. nov. (FIG. 1, 2, 3).

Maculae epiphyllae, ambitu, irregulari, 3–5 mm. in diam., centralibus atris nitensibus irregularibus stromatibus, 1–4 mm. diam., compositae, et flavo-brunnea zona circumdatu; loculi pauci in quoque stromate, applanati, 360–400 × 60–70 μ, in superiore parte crassi atri clypei et inferne nulli; asci clavati, octospori, 68–76 × 13–17 μ, breviter pedicellati; sporis stipati, continuis, hyalinis, longo-ellipsoideis, apicibus acuminatis, 19–23 × 8–9 μ; contextu granulato; paraphyses praesentes, filiformes, inconspicuae.



FIGS. 1–10.

Spots epiphyllous, irregular, 3–5 mm. in diam., composed of central black, shiny, irregular stromata, 1–4 mm. in diam., and a surrounding zone of yellow-brown tissue; locules few in each stroma, flattened, $360–400 \times 60–70\ \mu$, with a thick black clypeus above and none below; asci clavate, 8-spored, $68–76 \times 13–17\ \mu$, short stalked with spores crowded; ascospores 1-celled, hyaline, long-elliptical, with somewhat tapering ends, $19–23 \times 8–9\ \mu$, and context granular; paraphyses filiform, inconspicuous.

This species may be synonymous with *Phyllachora Copaiferae* Speg. from Peribebuy, Brazil. However, there seem to be some marked differences between the two; Spegazzini's species has cylindrical asci, $70–90 \times 10–12\ \mu$, and the spores are smaller, $16–18 \times 7–8\ \mu$.

On *Copaifera* sp., Itabituruna, *Josué Deslandes 2601*, Dec. 1934. Type.

72. CATACAUMA HAMMARI (P. Henn.) Theissen & Sydow, Ann. Myc. **13**: 389. 1915.

On *Machaerium* sp., Vicosa, *Muller 5*, Oct. 6, 1929.

73. CATACAUMA MUCOSUM (Speg.) Theissen & Sydow, Ann. Myc. **13**: 373. 1915.

Phyllachora mucosa Speg. Anal. Soc. Ci. Argent. **26**: 40. 1888.

The type specimen is *Balansa, Plantes du Paraguay 4069*, on *Cocos australis*, from Guarapi, Brazil. Our material matches well with the type. The long, graminicolous, amphigenous stromata are typical. They are shining black, well raised above the leaf sur-

FIGS. 1–3. *Catacauma copaiferiicola.* 1, leaf of *Copaifera* sp. showing groups of stromata in the epiphyll; 2, cross section of leaf of *Copaifera* sp. showing stromatic characters of the parasite; 3, an ascus and paraphyses and two enlarged ascospores. FIGS. 4–7. *Catacauma Tephrosiae.* 4, leaf of *Tephrosia* sp. showing pustular black stromata in the epiphyll; 5, an ascus, paraphyses, and two enlarged ascospores; 6, cross section of leaf of *Tephrosia* sp. showing the position of the stroma and locules; 7, enlarged microscopic view of fig. 6, showing details of stroma and its subepidermis position within the leaf tissues. FIGS. 8–10. *Phyllachora diocleicola.* 8, cross section of leaf of *Dioclea rufescens* showing uniloculate character of the parasite and the thick heavy clypeus above the locule; 9, portion of a leaf of *Dioclea rufescens* showing the epiphyllous circular spots made up of many punctiform stromata; 10, two asci with long pedicels, paraphyses and two ascospores enlarged.

face. In cross-section the stromata are subepidermal, enclosing 1–3 locules. Spores are elliptical, 18–24 × 8–10 μ, with a wall sometimes enclosed in a mucous sheath.

On *Cocos plumosa*, Vicosa, *Muller 647*, Dec. 7, 1933.

74. **Catacauma selenospora** (Speg.) Chardon, comb. nov.

Phyllachora selenospora Speg. Bol. Acad. Ci. Cordoba **11**: 544. 1889.

Catacauma semilunata Chardon, Jour. Dept. Agr. P. R. **13**: 7. 1921.

The Brazilian specimens show saccate asci, with eight arcuate, hyaline, 1-celled spores, 17–19 × 7–9 μ, distinctly half-moon shaped. This spore character agrees with *Phyllachora selenospora* Speg., the type of which has been examined. It is from Apiahy, Brazil, on Myrtaceae. The spores are of the same shape, 16–20 × 6 μ. The stromata are located between the epidermis and the mesophyll, and hence a new combination under *Catacauma* is here proposed.

Catacauma semilunata Chardon, from Maricao, P. R., seems to be the same species. *Catacauma Myrciae* (Lév.) Theissen & Sydow, with arcuate spores, and also on Myrtaceae may be the same, but the type has not been examined.

On *Britoa rugosa*, Lavras, *Deslandes 2604*, June 1934.

On *Myrtaceae*, Lagoa Santa, *Muller 964*, July 16, 1935.

On *Myrtaceae*, Curvello, *Muller 1031*, Mar. 1, 1936.

On *Myrtaceae*, Uberlandia, *Muller 1079*, May 17, 1936.

75. **Catacauma Tephrosiae** Chardon, sp. nov. (FIG. 4, 5, 6, 7).

TYPE: *Cornell Univ. Explor. Brazil 482.*

Stromata epiphylla, atra, prominentia, orbicularia vel irregularia in ambitu; 1 mm. in diam., dispersa, raro confluentia, partibus foliorum maculiformiter decoloratis, inter epidermidem et mesophyllum evoluta; loculi in superiore parte stromatis clypeis compositi, inferne nulla stromata, 1–3 in quoque stromate, applanati, 250–350 × 125–140 μ; asci clavati, sporis octonis, 75–95 × 21–27 μ, breviter pedicellati; sporis inordinatis vel stipatis, continuis, hyalinis, late-ellipsoideis, levis, 17–19 × 8–9 μ; paraphyses filiformes.

Stromata epiphyllous, black, prominent, circular, or irregular in outline, 1 mm. in diam., scattered, rarely coalescing, with discolored zone surrounding each stroma; stroma originating between epidermis and mesophyll, composed of heavy black clypeus above locules, with little on sides and none below; locules few, 1–3 in

each stroma, flattened, 250–350 × 125–140 μ; asci clavate, 8-spored, 75–95 × 21–27 μ, short stipitate, with spores inordinate, or crowded in ascus, 1-celled, hyaline, broad elliptical, smooth, 17–19 × 8–9 μ; paraphyses filiform.

The position of the stroma in the tissue of the leaves of the host tissue is clear; it originates between the epidermis and the mesophyll, not entering the inner tissue (mesophyll). The fungus is therefore described under *Catacauma*. There is no *Phyllachora*, or like fungus attacking *Tephrosia* in the New World.

On *Tephrosia* sp., Vicosa, *Muller 482*, Apr. 21, 1933.

76. CATACAUMA VENEZUELENSIS (Sydow) Chardon, Jour. Dept. Agr. P. R. **16**: 170. 1932.

Phyllachora venezuelensis Sydow, Ann. Myc. **28**: 107. 1930.

In our specimen the stromata are epiphyllous, black, approximately circular, 1–2 mm. in diameter. The asci are saccate, 72–89 × 15–17 μ, with the spores inordinate. Spores are elliptical to subglobose, 11–13 × 6–8 μ, hyaline or becoming light olivaceous with age. In the type material (Sydow's *fungi exot. exs. 830*), from Puerto La Gruz, Venezuela, the spores are subglobose, 10–16.5 × 9–12 μ.

On *Machaerium acutifolium*, Vicosa, *Muller 485*, Apr. 21, 1933, and *Muller 289*, Aug. 15, 1931.

77. **Phyllachora Acalyphae** Chardon, sp. nov.

TYPE: *Cornell Univ. Explor. Brazil 638.*

Stromata amphigena, atra, nitentia, ambitu, fere orbiculari, .5–.8 μ in diam., elevata supra superficiem folii; loculi 1–3, in mesophyllo immersi, lenticulares, magni, 340–435 × 200–280 μ, clypeo crasso, atro, amphigeno et stromatibus lateraliter praesentibus; asci paraphysati, octospori, cylindracei vel cylindraceo-clavati; 73–88 × 14–18, breviter pedicellati; sporae obliquae monostichae vel distichae, continuae, hyalinae, ellipsoideae, utrinque attenuatae, 14–17.5 × 7–8 μ, contextu homogeneo.

Stromata amphigenous, black, shining, approximately circular, .5–.8 μ in diam., raised above the leaf surface; locules 1–3 in each stroma, in mesophyll, lenticular, large 340–435 × 200–280 μ, with heavy black clypeus above and below and some stromatic tissue on sides; asci cylindrical to cylindric-clavate, 8-spored, 73–88 × 14–18 μ, short pedicellate, with spores obliquely uniseriate or partially biseriate; spores hyaline, 1-celled elliptical, tapering in ends,

14–17.5 × 7–8 μ, with the contents homogenous; paraphyses present.

On *Acalypha villosa,* Ana Florencia, Ponte Nova, *Muller 638,* June 21, 1933.

78. **Phyllachora anonicola** Chardon, sp. nov.
TYPE: *Cornell Univ. Explor. Brazil 584.*

Stromata amphigena, conspicua in epiphyllo, atra, nitentia, irregulariter elevata, maculas efficientea, 1–2 mm. in diam., minus in hypophyllo visibilia, applanata, atria, opaca; loculi 1–3 vel numerosi in quoque stromate, globosi, 180–250 μ in diam., in mesophyllo immersi, clypeo crasso, atro in parte superiore, et parietibus stromaticis; asci cylindracei vel cylindracei-clavati, 93–106 × 9–14 μ, octospori; sporis oblique monostichis vel sub-distichis, continuis, hyalinis, ellipsoidis, utrinque fusoidis, 12–13.5 × 5–6 μ; paraphyses praesentes.

Stromata amphigenous, conspicuous in the epiphyll, forming black shiny, irregular, slightly raised spots, 1–2 mm. in diam., less visible in the hypophyll, flat, dark, opaque; locules 1–3 or more in each stroma, globose, or nearly so, 180–250 μ in diam., located in the mesophyll with thick black clypeus above and stroma on the sides; asci cylindrical to cylindric-clavate, 93–106 × 9–14 μ, 8-spored, with spores obliquely uniseriate or partially biseriate, 1-celled, hyaline, elliptical, with one end tapering, 12–13.5 × 5–6 μ; paraphyses present.

This species differs markedly, both in stromatal and spore characters, from *Phyllachora atromaculans* Sydow, on *Anona* sp., from San Jose, Costa Rica, the type of which has been examined. It differs also from *Phyllachora Anonaceae* Rehm, from Sao Francisco, Brazil, the stromata of which are " in maculis hypophyllis."

On *Anona muricata,* Vicosa, *Muller 584,* June 4, 1933.

On *Anona muricata,* Serra da Grama, Carangola, *Drummond 916,* Apr. 12, 1935.

79. PHYLLACHORA BALANSAE Speg. Anal. Soc. Ci. Argent. **19**: 92. 1885.

The fungus appears in the form of numerous, punctiform, black stromata, covering an appreciable surface of the leaves of the host. Spores broad-elliptical, 10–12 × 7–9 μ. A common parasite on various species of *Cedrela* in continental South America.

On *Cedrela mexicana,* Vicosa, *Muller 669,* Dec. 30, 1933.

80. **Phyllachora diocleicola** Chardon, sp. nov. (FIG. 8, 9, 10).
TYPE: *Cornell Univ. Explor. Brazil 749.*

Maculae semper epiphyllae, fere orbiculares, 4–6 mm. in diam., numerosis, minutis, atris, clypeis compositae; loculi singuli, globosi vel lenticulares, 215–265 × 120–132 μ, in mesophyllo immersi, in superiore parte atri stromatici clypei, 50–60 μ crassi, supra superficiem folii, nullo stromate lateraliter et parvo inferne; asci cylindraceo-clavati, longi pedicellati, 68–81 × 13–14 μ. sporis octonis; sporis monostichis vel distichis, continuis, ellipsoidis, 13–15 × 7–9 μ, levibus, contextu granulato; paraphyses praesentes.

Spots epiphyllous, approximately circular, 4–6 mm. in diam., composed of numerous small black specks, the clypei of the locules; locule single, globose or lenticular, 215–265 × 120–132 μ, located in the mesophyll, with heavy black clypeus above, 50–60 μ thick, which protrudes above the leaf surface, no stromatic tissue on sides and little at base; asci cylindric-clavate, long pedicillate, 68–81 × 13–14 μ, 8-spored, with spores uniseriate or biseriate, 1-celled, lemon-shaped, 13–15 × 7–9 μ, smooth, with context granular; paraphyses present.

The species differs from *Phyllachora Diocleae* P. Henn., reported on the same host genus from Jurua-Mity, Brazil, in macroscopic stromatal characters and in having much smaller spores. In Henning's species, the spores are elongated (langlich), 20–23 × 6 μ.

On *Dioclea rufescens*, Vicosa, *Muller 749*, Feb. 18, 1934.

81. PHYLLACHORA CHLORIDICOLA Speg. Myc. Argent. **IV**, n. 706. 1909.

On *Chloris pycnochrix*, Vicosa, *Muller 27*, Nov. 20, 1929.

82. PHYLLACHORA ENGLERI Speg. Anal. Soc. Ci. Argent. **19**: 96. 1885.

A common species in the American tropics on members of the Araceae, especially species of *Anthurium*, producing black, circular, amphigenous, spots, 2–4 mm. in diameter.

On *Philodendron* sp., Lagoa Grande, *Muller 839*, Aug. 26, 1934.

83. **Phyllachora fusispora** Chardon, sp. nov.
TYPE: *Cornell Univ. Explor. Brazil 747.*

Stromata amphigena, atra, linearia, prominula, 1–2 × .5–.8 mm., leniter supra superficiem folii elevata; loculi 2–5 in mesophyllo immersis, applanato-globosi vel mutua pressione angulosi, 140–195 × 120–145 μ, membrana crassa atro-stromatica; asci octospori, cylindracei v. cylindraceo-clavati, 100–130 × 17–25 μ, sporis magnis, monostichis v. distichis, continuis, hyalinis, longo-fusoideis, 23–27 × 6–7 μ; paraphyses numerosae.

Stromata amphigenous, black, linear, conspicuous, 1–2 × .5–.8 μ, slightly raised above leaf surface; locules 2–5, flat, globose or angular through lateral pressure from others, immersed in mesophyll, black stromatic tissue on all sides, 140–195 × 120–145 μ; asci cylindric to cylindric-clavate, 8-spored, 100–130 × 17–25 μ, with spores uniseriate or biseriate, continuous, hyaline, long-fusoid, large, 23–27 × 6–7 μ, paraphyses numerous.

This species differs from all described *Phyllachorae* on *Andropogon* in the large spores and their fusoid shape.

On *Andropogon* sp., Vicosa, *Muller 747*, Feb. 18, 1934.

84. PHYLLACHORA INSULARIS Chardon, Jour. Dept. Agr. P. R. **13**: 11. 1929.

This species is common in the West Indies and northern South America on *Valota insularis*, a host which has also gone under the name of *Trichachne insulare*. The type is from Puerto Rico, *Whetzel and Olive 551*. The stromata in the Brazilian specimen are mostly epiphyllous, black, not shiny, arranged in a row parallel to the main axis of the leaf host. The locules, which are several in each stroma, show beautifully under the microscope; asci long-cylindrical, 72–76 × 8 μ, with the spores uniseriate, broad elliptical, 8–9 × 5–6 μ. All these characters agree with the type material and numerous collections from the West Indies.

This appears to be the first report of the species from Brazil. The host is also new for the parasite.

On *Trichachne sacchariflora*, Vicosa, *Muller 388*, July 21, 1932.

85. **Phyllachora Lundiae** Chardon, sp. nov. (FIG. 21, 22).

Stromata minuta, punctiforma, .2–.3 mm. in diam., numerosa, atria, in utraque foliorum pagina leniter prominula; loculi singuli, globosi, 185–220 × 108–132 μ, in superiore et inferiore parte atris clypeis compositi, nulli in lateribus; asci paraphysati, clavati, octospori, breviter pedicellati, 55–65 × 19–23 μ, sporis continuis, hyalinis, longis, ellipticis, utrinque acutis, levis, 18–23 × 5–6 μ, contextu granulato.

Stromata minute, punctiform, .2–.3 mm. in diameter, forming numerous black specks on both surfaces of leaf, slightly raised; locules single, globose, 185–220 × 108–132 μ, with black clypeus above and below and none on sides; asci clavate, 8-spored, short stipitate, small, 55–65 × 19–23 μ, with spores crowded in ascus, hyaline, long-elliptic with acute ends, smooth, 18–22 × 5–6 μ, contents granular; paraphyses present.

This is a minute unilocular *Phyllachora* such as would fall in Spegazzini's genus *Puiggarina* (created to include the uniloculate species of *Phyllachora*). There is no evidence of a perithecial wall being present, but small black clypei are found bordering the top and bottom of the locules. Hence the fungus is retained under *Phyllachora*. The small size of the asci, their clavate shape, the crowded arrangement of the spores, and the shape of the spores are distinct specific characters of this new species.

On *Lundia longa*, Vicosa, *Muller 464*, Apr. 16, 1933.

86. **Phyllachora mabaeicola** Chardon, sp. nov.

TYPE: *Cornell Univ. Explor. Brazil 576.*

Stromata amphigena fere, orbiculares, 1 mm. in diam., leniter in epiphyllo elevata; loculi 2–5 in quoque stromate, applanato-lenticulares, in mesophyllo immersi, 215–252 × 132–156 μ, clypeo in superiore et in inferiore parte, lateraliter nonnullis stromaticis praesentibus; asci cylindracei, octospori, 85–94 × 14–17 μ, sporis monostichis, continuis, hyalinis, globosis, 9–11 μ, membrana tenui, contextu homogeneo; paraphyses praesentes.

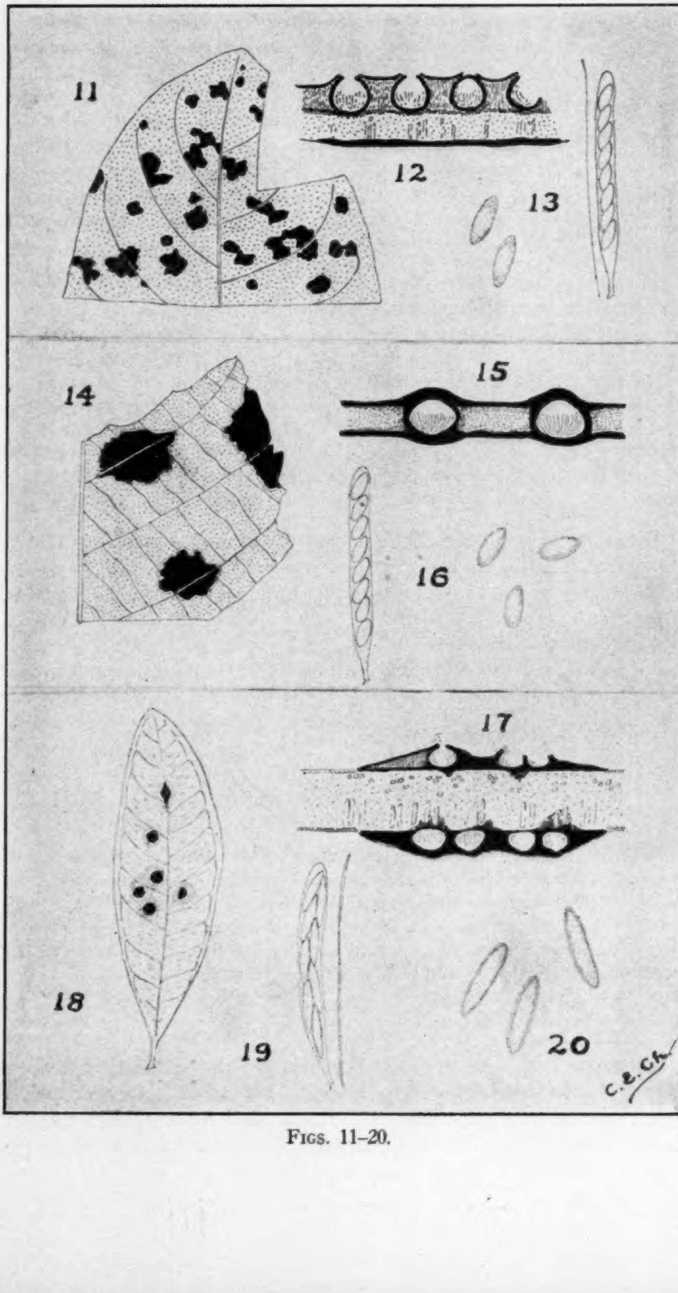
Stromata amphigenous, approximately circular, 1 mm. in diam., numerous, brown, slightly raised in epiphyll; locules 2–5 in each stroma, flat-lenticular, immersed in mesophyll, 215–252 × 132–156 μ, with clypeus above and below and some on sides; asci cylindric, 8-spored, 85–94 × 14–17 μ, with spores uniseriate, continuous, hyaline, globose, 9–11 μ with thin walls, contents homogenous, paraphyses present.

On *Mabaea fistulifera*, Vicosa, *Muller 576*, June 3, 1933.

87. **Phyllachora macroloculata** Chardon, sp. nov. (FIG. 11, 12, 13).

Stromata amphigena, atra, magna, conspicua, irregularia, vel angulata in ambitu, 5–8 mm. in diam., superne verrucoso-papillatis loculis, levia et atra in hypophyllo; loculi numerosi in epiphylli stromate, globosi vel subglobosi, 300–350 × 240–265 μ, atris stromatibus cincta, in superiore parte tenui clypeo, stromatica membrana inter loculo, subhyalina vel dilutissime griseo-brunneolo, et stromate in hypophyllo reducto; asci longo-cylindracei, octospori, 65–78 × 7–9 μ, sporis oblique distichis, continuis, hyalinis, longo-naviculare, 14–17 × 5–6 μ, leves; paraphyses praesentes.

Stromata amphigenous, dull black, large, conspicuous, irregular or angular in outline, 5–8 mm. in diam., surface in epiphyll roughened with papillate locules, smooth and dull black in hypophyll; locules many in epiphyllous stromata, globose or nearly so, 300–



FIGS. 11–20.

350 × 240–265 μ, bordered with black stroma, with thin clypeus above and stromatic tissue between locules of much lighter color and composed of vertical elongated cells and the stroma in the hypophyll reduced to a simple, black crustlike clypeus; asci long-cylindric, 8-spored, 65–78 × 7–9 μ, with spores obliquely biseriate; spores continuous, hyaline, long-navicular, 14–17 × 5–6 μ, smooth; paraphyses present.

The stromata in this species form large, conspicuous, irregular, black spots, visible on both leaf surfaces, tar-like in appearance. The epiphyllous stromata form rather complex structures: a thin, black clypeus on the top, a black border of the individual locules and a layer of much lighter colored long cells in the interlocular region. All of these parts show very plainly under the microscope.

On *Guettarda* sp., Araponga, *Muller 341*, Apr. 30, 1934.

88. **Phyllachora magnificens** Chardon, sp. nov. (FIG. 14, 15, 16). TYPE: *Cornell Univ. Explor. Brazil 178.*

Stromata amphigena, atra, magna, conspicua, in ambitu angularia, 1–3 cm. in diam., superne verrocosis minutibus papillatis ostiolis; maculae tenui grisea membrana circumdatae; loculi numerosi, globosi vel applanati, 265–325 × 190–230 μ, crassis atris clypeis superne et inferne; asci long-cylindracei, octospori, 105–120 × 8–9 μ, sporis oblique monostichis, continuis, hyalinis, longo-ellipsoideis, apicibus attenuatis, 10–13 × 5–6 μ; paraphyses filiformes.

Stromata amphigenous, black, large, conspicuous, angular in outline, 1–3 cm. in diam., surface roughened with minute papillate ostiola; spots bordered with a thin whitish tissue; locules numerous, globose or flattened, 265–325 × 190–230 μ, with thick black clypei above and below; asci long-cylindric, 8-spored, 105–120 × 8–9 μ, with spores obliquely uniseriate, continuous, hyaline, long-elliptical, with one end sometimes tapering, 10–13 × 5–6 μ; paraphyses filiform.

FIGS. 11–13. *Phyllachora macroloculata.* 11, portion of leaf of *Guettarda* sp. showing conspicuous, black, irregular stromata in the epiphyll; 12, cross section of fructification showing group of epiphyllous locules and the stromatic band in the hypophyll; 13, an ascus, paraphyses, and two enlarged ascospores. FIGS. 14–16. *Phyllachora magnificens.* 14, fragments of a leaf of *Apeiba* sp. showing large size of stromata; 15, cross section of leaf and stroma showing the locules and their position within the host tissue; 16, an ascus and three enlarged ascospores. FIGS. 17–20. *Phyllachora Mulleri.* 17, cross section of leaf of *Eugenia dodonaefolia* showing stromata, locules and their relation with the host tissue; 18, leaf of *Eugenia dodonaefolia,* showing conspicuous stromata in the epiphyll; 19, an ascus and paraphysis; 20, three enlarged ascospores showing wall.

This is a handsome specimen of *Phyllachora*, with very large, black angular spots, visible on both sides of the leaves. The locules are numerous on each spot, appearing as numerous, prominent specks. The species is apparently new to science, being different from everything the writer has seen, and well deserves the specific name *magnificens*.

On *Apeiba* sp., Vicosa, *Muller 178*, May 29, 1930.

89. PHYLLACHORA MALABARENSIS Sydow & Butl. Ann. Myc. **9**: 398. 1911.

The spores of this species are unusually large for the genus, 27–35 × 9–14 μ, which do not fit any of the *Phyllachorae* described on *Bambusa* from South America. The writer has not seen the type of *malabarensis* to make a comparison, but the stromatal as well as spore characters are well within the diagnosis.

On *Bambusa* sp., Vicosa, *Muller 113*, Dec. 20, 1929.

90. **Phyllachora Mulleri** Chardon, sp. nov. (FIG. 17, 18, 19, 20). TYPE: *Cornell Univ. Explor. Brazil 851.*

Stromata amphigena, orbicularia vel irregularia, conspicua, atra, nitentia. 1.5–3 mm. in diam., ad superficium ob numerosos loculos papillate, zonulis violaceis cincta, in cuticulo evoluta et aetate in mesophyllum penetrantia; loculi numerosi in quoque stromate, 5–15 v. plures, globosi, apicis leniter conicis et in inferiori parte applanati, 285–360 × 218–260 μ, atro stomate circumdati; asci cylindracei vel cylindraceo-clavati, octospori, 95–120 × 13–15 μ, sporis inordinatis vel distichis, longo-fusoideis, continuis, hyalinis, 28–32 × 6–7 μ, utrinque subacutis, contextu granulato; paraphyses filiformes.

Stromata amphigenous, circular, or irregular, conspicuous, black, shiny, 1.5–3 mm. in diam., surface papillate from numerous locules, surrounded by a violet zone of host tissue; stromata originating under the cuticle and at maturity extending into the mesophyll; locules numerous in each stroma, 5–15 or more, globose, slightly conical at apex and flattened at base, 285–360 × 218–260 μ, surrounded by black stroma; asci cylindric to cylindric-clavate, 8-spored, 95–120 × 13–15 μ, with spores inordinate or biseriate in the ascus, spores long-fusiform, 1-celled, hyaline, 28–32 × 6–7 μ, with ends sub-acute and contents granular, paraphyses filiform.

This is an interesting and beautiful species, having conspicuous, black stromata equally visible on both surfaces of the leaf. A cross section of the leaf shows the stromata with numerous locules, sub-cuticular (like a *Trabutia*), but the stromatic tissue sometimes

penetrates the inner leaf tissue, so it is described as a *Phyllachora*.

The spores are long fusiform, with measurements like those of *Phyllachora Petitmengini* Maire, which is known on Myrtaceae from Sao Paulo, Brazil. In stromatal characters our species seems to be very distinct. It is dedicated to its collector, the junior author.

On *Eugenia dodonaefolia* Teixeras, *Muller 851*, Oct. 26, 1934.

91. PHYLLACHORA PANICI (Rehm) Theissen & Sydow, Ann. Myc. **13**: 452. 1915.

Physalospora Panici Rehm, Hedwigia **40**: 114. 1901.

This species was described from the general locality of this collection. It is distinct from other species on *Panicum* in the small spores—7–9 × 5 μ.

On *Panicum sciurotes*, Vicosa, *Muller 489*, Apr. 22, 1933.

92. PHYLLACHORA PARAGUAYA Speg. Anal. Soc. Ci. Argent. **19**: 243. 1883.

On *Luhea divaricata*, Teixaras, Vicosa, *Muller 2*, Oct. 6, 1929.
On *Luhea* sp., Uberlandia, *Muller 1057*, May 18, 1936.

93. PHYLLACHORA PAZSCHKEANA Sydow, Bull. Herb. Boiss. 80. 1901.

This differs from the above species on *Panicum* in possessing larger spores—10–14 × 6–7 μ.

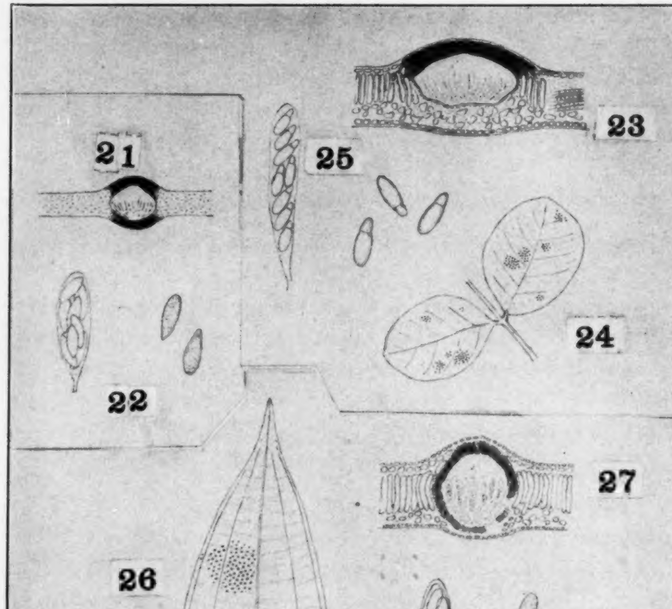
On *Panicum* sp., Sao Miguel, *Muller 328*, Mar. 19, 1932.

94. PHYLLACHORA PSYCHOTRIAE Rehm, Hedwigia **36**: 371. 1897.
On *Psychotria hancorniifolia*, Vicosa, *Muller 796*, May 20, 1934.

95. **Phyllachora phylloplaca** Chardon, sp. nov. (FIG. 35, 36, 37).
TYPE: *Cornell Univ. Explor. Brazil 491.*

Maculae amphigenae, conspicuae, atria, nitentea, angulares, 3–5 mm. in diam.; loculi numerosi, minuti, 90–120 μ in diam., in mesophyllo immersi, superne atro clypeo et parvo stromate inferne; asci clavati, 8-spori, 55–63 × 13–15 μ, breviter stipitati; sporis distichis vel inordinatis, hyalinis, continuis, oblongo-ellipsoideis, postice attenuatis, 9–11 × 4–5 μ, levibus; paraphyses praesentes.

Spots amphigenous, conspicuous, black shiny, angular, 3–5 mm. in diam., locules many, small, 90–120 μ in diam., immersed in the mesophyll, black clypeus above and thin one below; asci clavate,



FIGS. 21–31.

8-spored, 55–63 × 13–15 μ, short stipitate, with spores biseriate or inordinate; spores hyaline, one-celled, long-elliptical, with one end tapering, 9–11 × 4–5 μ, smooth; paraphyses present.

On *Diclidanthera laurifolia*, Vicosa, *Muller 491*, Apr. 22, 1933.

95. PHYLLACHORA PUSILLA Sydow, Ann. Myc. **2**: 163. 1904.

On *Malvaceae*, Vicosa, *Muller 585*, June 4, 1933.

96. PHYLLACHORA SCLERIAE Rehm, Hedwigia **39**: 232. 1900.

The type specimen is from Maua, Rio Janeiro. It has not been examined, but the material agrees well with the published description and with material from the West Indies which has been referred to *Phyllachora Scleriae*. The asci are cylindrical, with the spores biseriate. The spores long-fusiform with sub-acute ends, and the dimensions, 21–25 × 5–6 μ, are slightly larger than Rehm's, which are 18–22 × 4–4.5 μ.

On *Scleria* sp., Vicosa, *Muller 427*, Mar. 29, 1933.

97. PHYLLACHORA SECURIDACAE P. Henn. Hedwigia **43**: 251. 1904.

On *Polygalaceae*, Vicosa, *Muller 544*, May 21, 1933.

98. PHYLLACHORA SPHAEROSPERMA Winter, Hedwigia **21**: 170. 1884.

Common throughout tropical America. Spores uniseriate or biseriate, globose, 8–9 μ in diameter.

On *Cenchrus* sp., Rio Branco, *Muller 829*, July 21, 1934.

99. PHYLLACHORA TARUMA Speg. Anal. Soc. Ci. Argent. **19**: 94. 1886.

On *Vitex cymosa*, Vicosa, *Muller 166*, Apr. 24, 1930.

FIGS. 21–22. *Phyllachora Lundiae*. 21, cross section of stromata showing uniloculate character and its relation to the host tissue; 22, an ascus and two enlarged ascospores with sub-pyriform shape. FIGS. 23–25. *Stigmochora controversa*. 23, cross section of stroma; 24, leaves of *Menoxylon brauna* (Muller *691*) showing groups of minute stromata in the epiphyll; 25, an ascus and three enlarged ascospores. FIGS. 26–28. *Guignardia atropurpurea*. 26, portion of leaf of *Miconia* sp. showing typical spot with group of perithecia; 27, cross section of perithecium with wall; 28, ascus and two enlarged ascospores. FIGS. 29–31. *Guignardia punctiformis*. 29, a portion of leaf of *Miconia* sp. showing circular spots produced by the parasite, filled with minute punctiform stromata; 30, cross section of leaf of *Miconia* showing single perithecium with clypeus; 31, an ascus and two enlarged ascospores.

On *Vitex cymosa*, Maria da Fe., Minas, *Muller 225*, Dec. 29, 1930.

100. PHYLLACHORA TROPICALIS Speg. Anal Soc. Ci. Argent. **10**: 143. 1880.

The type specimen is from Cordoba, Argentine, on *Psidium Theae*. The stromata are much smaller than in the Brazilian collections, amphigenous, .5–1 mm. in diameter, embedded in the mesophyll; the spores uniseriate, elliptical, 12–14 × 7–8 μ.

In our number *528*, the stromata are much larger, only epiphyllous, black, shining, circular, raised over the surface of the leaf, 1–2 mm. across. In cross-section, microscopically, the stromata are distinctly sub-epidermal (suggestive of *Catacauma*) with large locules, 500–600 × 240–275 μ; asci cylindrical-clavate, the spores obliquely uniseriate or partially biseriate; spores elliptical, with blunt ends, 15–18 × 7–8 μ, with the contents granular; paraphyses present.

In spite of the apparent differences between the specimen the writers do not feel justified in considering it as a new species. The material collected by the junior author compares well with *Puttemans Fungi 170*, from Serra da Cantareira, Sao Paulo, determined by Puttemans as *Phyllachora tropicalis*; also with a specimen collected by Maublanc in Bello Horizonte, Minas Geraes, which has been referred to the same species. In both of these two specimens the stromata are much larger than in the type specimen.

On *Myrtaceae*, Ouro Preto, *Muller 528*, May 2, 1933.

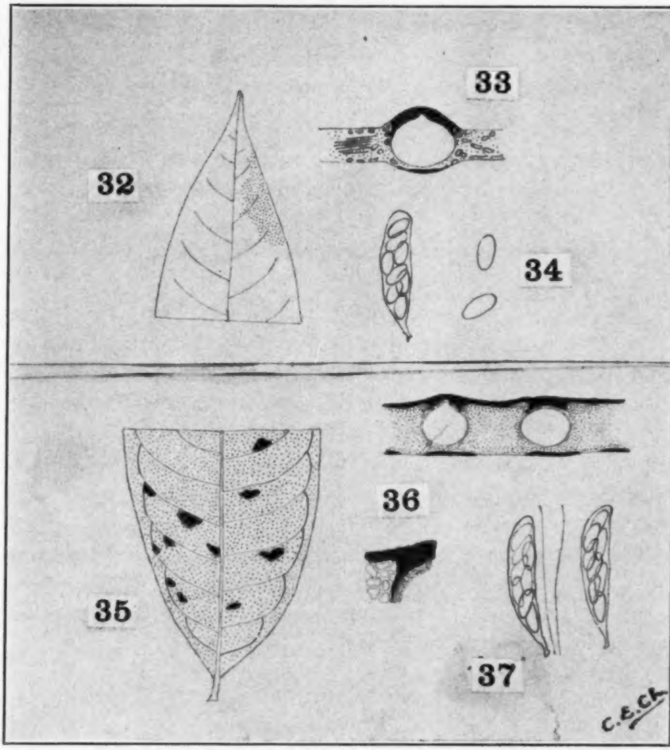
On *Psidium guajava*, Vicosa, *Muller 823 & 824*, July 11, 1934.

101. PHYLLACHORA VERNONIICOLA P. Henn. Hedwigia **41**: 110. 1902.

On *Vernonia* sp., Vicosa, *Muller 414*, Mar. 22, 1933.

102. **Phyllachora Vicosae** Chardon, sp. nov. (FIG. 32, 33, 34). TYPE: *Cornell Univ. Explor. Brazil 751.*

Stromata amphigena, minuta, punctiforma, inconspicua, .2–.3 mm. in diam., sine maculis, in folii late dispersa, atra in epiphyllo, brunnea in hyphyllo; loculi singuli, globosi, vel applanati, minuti, 125–246 × 68–127 μ, atro clypeo superne et parvo inferne; asci clavati, octospori, 58–70 × 12–14 μ, breviter pedicillati, sporis inordinatis, hyalinis, continuis, longo-ellipsoideis, utrinque obtusis, 12–14 × 5 μ; paraphyses praesentes.



FIGS. 32–34. *Phyllachora Vicosae.* 32, portion of leaf of *Machaerium* sp. invaded with a large number of minute stromata of the parasite; 33, cross section of leaf of *Machaerium* sp. showing uniloculate character of the stroma and the clypeus; 34, an ascus and two enlarged ascospores. FIGS. 35–37. *Phyllachora phylloplaca.* 35, a portion of leaf *Diclidanthera laurifolia* showing typical *Phyllachora*-like spots of the parasite; 36, cross section of perithecia showing heavy black clypeus above and a smaller stromatic band below; 37, two asci and paraphyses.

Stromata amphigenous, small, punctiform, inconspicuous, .2–.3 mm. in diam., covering wide areas of the leaf, black in epiphyll, brown in hypophyll; locule single, globose or flattened, small, 125–240 × 68–127 μ, with black clypeus above the locule and smaller one below; asci clavate, 8-spored, 58–70 × 12–14 μ, short stipitate with spores inordinate; spores hyaline, one-celled, long-elliptic with ends obtuse, 12–14 × 5 μ, paraphyses present.

The distinction between this species and the other on *Machae-*

rium, lies in the spores—12–14 × 5 μ here, and 5–6 × 3μ in *Phyllachora machaeriicola* (P. Henn.) Th. & Sydow.

On *Machaerium* sp., Vicosa, *Muller 751,* Feb. 18, 1934.

103. STIGMOCHORA CONTROVERSA (Starb.) Theissen & Sydow, Ann. Myc. **13**: 580. 1915.

Apiospora controversa Starb. Ark. Bot. **5**^{7}: 22. 1905 (FIG. 23, 24, 25).

Dothidella controversa Speg. Anal. Mus. Nac. Buenos Aires **23**: 95. 1912.

The stromata are punctiform, epiphyllous, black, crowded in dense groups which form spots 3–6 mm. in diameter. In cross-sections, the locules are embedded in the mesophyll, with a thick, heavy, black clypeus above and none on the sides. The asci are cylindrical-clavate, 8-spored with the spores partially biseriate in the ascus. The spores are hyaline, unequally 2-celled, 17–19 × 6–7 μ, with the lower cell much smaller, papillate; paraphyses present.

The type specimen has not been examined, but the characters agree closely with Theissen and Sydow's description. The species seems to have a wide distribution in South America, occurring on various leguminous hosts.

On *Menoxylon brauna,* Vicosa, *Muller 691,* Feb. 4, 1934.

On *Tecolobium* sp., Vicosa, *Muller 995,* Nov. 2, 1935.

104. RHOPOGRAPHUS ZEAE Pat. Bull. Soc. Myc. Fr. **9**: 136. 1893.

There is a linear stroma sunken in the corn culm, with a few locules in a single row, surrounded by bright colored stromatic tissue; with asci 90–130 × 16–18 μ, with filiform paraphyses, and ascospores cylindric-spindle form, constricted in the middle, 35–40 × 6–8 μ, 4–6 celled, brown.

On *Zea mays,* Vicosa, *Muller 1107,* Mar. 18, 1936.

105. RHOPOGRAPHUS BAMBUSAE (Cooke) Theissen & Sydow, Ann. Myc. **13**: 426. 1915.

On *Bambusa pallescens,* Serra da Grama, Carangola, *Drummond 930,* Apr. 12, 1935.

MICROTHYRIALES

106. ASTERINA MICONIAE Theissen, Ann. Myc. **11**: 440. 1913.

On *Miconia* sp., Carangola, *Muller 911,* Apr. 12, 1935.

107. **Coscinopeltis Tetrapteridis** Chardon, sp. nov.
TYPE: *Cornell Univ. Explor. Brazil 814.*

Maculae semper epiphyllae, plus-minusve orbiculares, 1.5–2.5 mm. in diam., atrae conspicuae, crustaceae, subcuticularibus stromatibus compositae; loculi numerosi in quoque stromate, applanati 400–600 × 210–214 μ in superior parte clypei, inferne nulla stroma, ostiolati; asci saccati, octospori, breviter pedicellati, apicis crassis (5 μ), et sporis biseriatis vel inordinatis, continuis, hyalinis, longe-pyriformis, utrinque arctuatis, 13–17 × 4.5–6 μ; paraphyses uncinatae, apicibus crassis.

Spots epiphyllous, more or less circular, 1.5–2.5 mm. in diam., composed of black, conspicuous, crustlike, subcuticular stromata; locules numerous in each stroma, flat, 400–600 × 210–214 μ, with clypeus above and none below, ostiolate; asci saccate, 8-spored, short pedicellate, apex thick (5 μ), and spores biseriate or inordinate; spores 1-celled hyaline, long-pyriform, with one end arcuate, 13–17 × 4.5–6 μ; paraphyses coiled and thickened at tips.

A cross-section of the fructification shows the stroma to be clearly sub-cuticular, so the fungus belongs in the tribe Munkielleae of the Polystomellaceae. In this tribe, *Coscinopeltis* has 1-celled, hyaline spores and paraphyses. Only two species have been described in this genus, from both of which our material differs widely.

On *Tetrapterix* sp., Vicosa, *Muller 814,* June 2, 1934.

108. **Ellisiodothis Qualeae** Chardon, sp. nov.
TYPE: *Cornell Univ. Explor. Brazil 963.*

Stromata hypophylla, carbonacea, tuberculata, suborbicularia, conspicua, 1–2 mm. in diam., ad superficiem folii adnata; stromata verrucosa minutissime papillata ostiolata, ad folium hypostromate centrali affixa, 120–150 μ lata, penetrans mesophyllum; loculis 2–5 in quoque stromate, sub-globosis, 225–300 μ in diam., ascis cylindraceis octosporis 90–105 × 8–11 μ; sporis oblique monostichis, continuis, hyalinis, oblongis, ellipsoideis, 8–11 × 5–6 μ, utrinque attenuatis; paraphyses praesentes.

Stromata hypophyllous, carbonous, tuberculate, nearly circular, conspicuous, 1–2 mm. in diameter, closely adnate to the surface of the leaf, with surface of stroma minutely roughened with protruding ostiola, attached to leaf with central hypostroma, 120–150 μ wide, which penetrates the mesophyll; locules 2–5 in each stroma, globose or nearly so, 225–300 μ in diam.; asci cylindric, 8-spored, 90–105 × 8–11 μ; spores obliquely uniseriate, continuous, hyaline, long-elliptical, 8–11 × 5–6 μ, with one end sometimes tapering; paraphyses present.

This species fits well in the genus *Ellisiodothis*, of the tribe *Polystomelleae* (which has round locules attached to the host by means of an intramatricular hypostroma). *Ellisiodothis* is the only genus in this tribe having 1-celled, hyaline spores and paraphyses.

On *Qualea multiflora*, Lagoa Santa, *Muller 963*, July 16, 1935.

109. LEMBOSIA MELASTOMATUM Mont. Ann. Sci. Nat. IV. **5**: 373. 1856.

On *Miconia Mendoncaei*, Vicosa, *Muller 580*, June 2, 1933.

110. ECHIDNODES BACCHARIDINCOLA (Rehm) Theissen & Sydow, Ann. Myc. **15**: 422. 1917.

On *Baccharis* sp., Ouro Preto, *Muller 529*, May 2, 1933.

111. RHAGADOLONIUM CUCURBITACEARUM (Rehm) Theissen & Sydow, Ann. Myc. **12**: 275. 1914.

On *Cayaponia* sp., Vicosa, *Muller 305*, Mar. 5, 1932.

HELOTIALES

112. DISCOHAINESIA OENOTHERAE (Cooke & Ellis) Nannf. Nova Acta Reg. Soc. Sci. Upsal. IV. **8**: 88. 1932.

Peziza Oenotherae Cooke & Ellis Grevillea **6**: 90. 1878.

None of the many different names applied to this fungus, such as *Pezizella Lythri* (Desm.) Shear & Dodge, are correct because they are based on a conidial stage.

On *Fragaria chiloensis*, Vicosa, *Muller 242*, Feb. 26, 1931.

113. PHAEOFABRAEA MICONIAE Rehm, Ann. Myc. **7**: 541. 1909.

This discomycete is a parasite on the stroma of *Bagnisiopsis tijucensis* Theissen & Sydow. The spores are brown, 2-celled, $10–14 \times 6–7\ \mu$. The type was collected in Sao Leopoldo, Brazil.

On *Miconia Candolleana*, Vicosa, *Muller 606*, June 6, 1933.

TAPHRINALES

114. TAPHRINA DEFORMANS (Berk.) Tul. Ann. Sci. Nat. V. **5**: 122–236. 1866.

On *Prunus Persica*, Itajube, *Muller 1133*, Oct. 15, 1936.

STUDIES ON TWO STRAINS OF APHANOMYCES LAEVIS FOUND OCCURRING AS WOUND PARASITES ON CRAYFISH [1]

RALPH I. SMITH [2]

(WITH 1 FIGURE)

During 1937 and 1938 a number of young crayfish (*Cambarus Clarkii* Girard) which had recently been operated upon in these laboratories [3] died, apparently as the result of infection with a water mould which after isolation was tentatively identified as *Aphanomyces laevis* De Bary. Since this species is not very clearly defined, and has never been reported as parasitic upon animals, and because of the fact that a severe, recurring epidemic disease of European crayfish is caused by a species of *Aphanomyces* (*A. astaci* = *magnusi* Schikora), which is, however, quite different from *A. laevis*, the following studies were made to determine more carefully the morphology, development, and taxonomic position of the present fungus.

Two strains, A and B, believed to be *A. laevis* have been isolated, each from a different lot of crayfish. These isolates, although morphologically very similar, show important physiological differences which are of interest in view of the varying descriptions of *A. laevis* that have previously been given.

The hyphae of both strains are slender and whitish, rather straight, with occasional side branches given off nearly at right

[1] Contribution No. 180 from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University.

[2] I am greatly indebted to Miss P. F. Sullivan and Dr. John R. Raper for their kind help in the preliminary isolation and identification of the fungus here discussed. To Dr. W. H. Weston, Jr., under whose direction the body of this work has been done, I am especially grateful for much advice and encouragement.

[3] The geographical source of the fungus to be discussed is not known. The crayfish infected with it were hatched at Cambridge, Massachusetts, from the eggs of crayfish shipped from Louisiana, but were kept for a time in tanks previously occupied by animals from other distant areas.

angles to the main axis. The diameter of hyphae grown on Difco cornmeal agar is from 3.5 μ to 8.5 μ, averaging 6 μ. The hyphae taper only slightly, the tips being bluntly rounded. The mycelial growth of both strains on most types of nutrient agar is submerged and similar, the radial growth rate of both on Difco cornmeal agar, for example, being about 0.9 cm. per day at room temperature. However, on unstrained cornmeal agar, strain A shows a sparse aerial growth, while strain B develops an aerial growth sufficiently dense to conceal the substrate.

Asexual reproduction in both strains is typical of the genus. The sporangia appear to be undifferentiated hyphae, sometimes branched, delimited proximally by a hyaline plug. The encysted zoöspores are 8–11.5 μ in diameter, remaining in clusters at the mouths of the long sporangia. Zoöspores are often left in the sporangium as cylindrical bodies of various lengths which may germinate in situ. Especially in old cultures, there is a pronounced tendency for the hyphal contents to aggregate to more or less elongate, sometimes branched, bodies which can sprout when placed in fresh water. When pieces of old agar cultures are placed in fresh water, slender sporangia form, which discharge small masses of 6–20 encysted spores. Larger sporeballs, such as are common in the genus, containing 100 or more spores, are usually produced by strain B when it is grown on hempseed in water, but almost never by strain A. The encysted primary zoöspores may germinate directly, or may release laterally biflagellate secondary zoospores which settle down and develop, usually by unequal bipolar germination.

Sexual reproduction is by means of antheridia and oögonia, which in strain A are formed rapidly and in great numbers on hempseed in water and on unstrained cornmeal agar, but never on Difco cornmeal agar. Strain B, on the other hand, forms oögonia only very rarely under any conditions. The oögonia of the two strains cannot be distinguished from each other, either in size or in general appearance (FIG. 1). In its scanty and slow production of oögonia, strain B is very similar to Coker's (**1**) typical form of *A. laevis*, but the rapid and abundant production of oögonia by strain A marks it as different from Coker's typical form in this respect.

Oögonia are formed singly or in small clusters, antheridial branches investing them in a loose tangle in which the connections are largely obscured. The antheridia appear to be of diclinous origin, but because most oögonia are formed on cloudy or opaque solid media it cannot be stated that these strains are invariably diclinous. The oögonial stalk sometimes winds closely about an antheridial branch, a condition reported by Drechsler (**3**) for *A. cladogamus* and *A. camptostylus*.

The oögonia are globose, 20–35 μ, averaging 28 μ, in diameter, with a smooth hyaline wall about $\frac{3}{4}$ μ thick. Because of the refractive nature of this wall, it is difficult to measure in optical section, but many observations have led to the conclusion that it is of uniform thickness, without the sinuous inner contour found in several species of *Aphanomyces* occurring as root parasites (Drechsler, **3**). The septum of the oögonial stalk is seldom observed because of the intertwining of the oögonial and antheridial branches. In all cases in which the septum has been seen, it has been found to lie not at the point where the stalk meets the globe of the oögonium, but at a distance usually as great as or greater than the radius of the globe (FIG. 1).

The single round oöspore is 16–29 μ in diameter, averaging 22 μ, with a smooth, non-refractive wall about 1 μ thick, which in any given oögonium is almost always thicker than the oögonial wall.

The oöspore wall is not quite hyaline, having a faint brownish or purplish tinge. The content of the oöspore is yellow-brown to olive with a regularly granular peripheral cytoplasm enclosing a large, slightly eccentric oil-drop. A small, clear vesicle is usually found lying in the thickest part of the granular area (FIG. 1). There is a tendency for the oögonia to be slightly larger when grown on solid nutrient agar than when developed on hempseed in water, but the appearance is very similar in all cases.

A feature which is not satisfactorily measured, but which is of taxonomic importance is the form of the antheridium. In my isolates there are one to three antheridia per oögonium, of the shape typical for *A. laevis,* that is, elongate, rather vermiform and constricted at intervals so as to rest upon the oögonium at several points like stout caterpillars (FIG. 1). The diameter increases gradually from the septum, the extreme end being bluntly rounded.

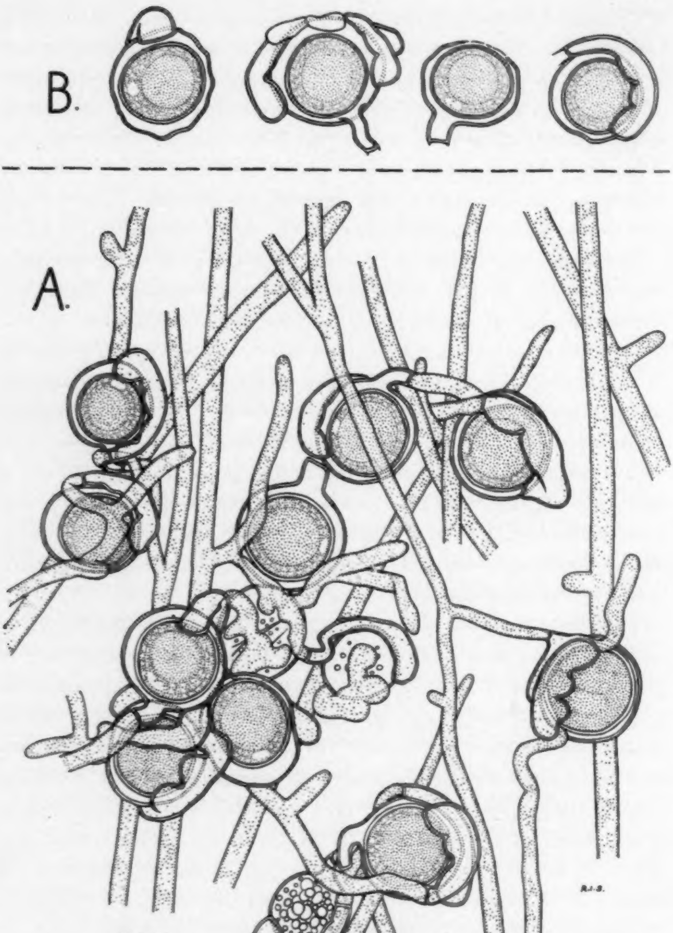


FIG. 1. The sexual organs of *Aphanomyces laevis*, grown in unstrained cornmeal agar cultures and drawn in the living condition with the aid of a camera lucida, at an original magnification of 1000 ×. The present magnification is about 550 ×, the exact measurements being shown in the scale, which applies to both (*A*) and (*B*). *A*. Oogonia, oospores, antheridia, and hyphae of strain A, drawn in situ, slightly simplified. The abundant for-

The antheridia differ, therefore, from the short, globose antheridia, or those with apical prolongations such as are found in the several soil inhabiting *Aphanomyces* figured by Drechsler (**3**).

That strain A is not homogeneous is indicated by the fact that from this strain cultural lines have been derived which produce no oögonia, but until further work has been done, no explanation can be offered. Of two single-spore lines of strain A, one has produced oögonia, the other has not.

The ability of strains A and B to infect wounded crayfish is probably not greatly different. All the animals naturally infected with strain A died, but attempts to inoculate healthy crayfish with it gave such indefinite results that I decided it was merely a wound parasite which could bring about the death of a weakened animal by preventing wound healing and increasing the chance of further infection by other organisms. Strain B showed little tendency to spread from animal to animal and was usually not fatal, but this lack of virulence may be attributed as much to the excellent health of the crayfish at that time and the prompt attempts to check the spread of the infection as to any lesser degree of infectiousness in this strain.

The appearance of the sexual organs (FIG. 1), and the measurements summed up in table I make it clear that both strains A and B can be assigned to the species *Aphanomyces laevis.* Several previously published measurements of this species, also included in table I, show that the species, as defined in the literature, is somewhat variable. A few words of explanation regarding several points in the table are necessary. As De Bary's (**2**) measurements are given in lines ($\frac{1}{12}$ inch; ‴), the values marked with an asterisk are taken from Peters (**7**), who converted De Bary's values into micra. The value marked with a (†) for diameter of oögonia after De Bary is Coker's (**1**) conversion of De Bary's measurements. Coker states that this is De Bary's measurement of the "eggs" of *A. laevis*, but as De Bary gives values only for

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mation of sexual organs is characteristic of this strain. *B*. Several oogonia, oospores, and antheridia of strain B. These are rarely formed, but may be seen to be in all respects similar to those of strain A.

TABLE I

THE CHARACTERISTICS OF *A. laevis* AND *A. astaci*, TAKEN FROM SEVERAL PREVIOUS AUTHORS FOR COMPARISON WITH THOSE OF THE STRAINS DISCUSSED IN THIS PAPER

Species	Worker	Occurrence	Diameter of hyphae	Diameter of sporangia	Diameter of encysted zoöspores	Diameter of oögonia	Diameter of oöspores	Oögonial wall	Oöspore wall	Antheridia
A. laevis	De Bary (2)	Dead insects in water	*5.3μ	*6.5μ	*9.7μ	*26.3–31.4μ †27–33μ	—	Thin, smooth	—	Elongate, rather lobed, androgynous or diclinous
A. laevis	Coker (1)	Saprophytic	5–7.5μ	Same as hyphae	7.3–11μ	18–33μ	16.5–26μ	Thin, smooth	‡"Thick"	Abundant, cylindrical, lobed, androgynous or diclinous
A. laevis	Coker (1)	Parasitic on desmids and diatoms	3–6.6μ	Typical	8.5–10μ	22–32μ	14–19μ	Smooth, sinuous	—	Elongate
A. laevis	Peters (7)	Parasitic on beets	5–9(10)μ	—	8–10.8μ	18.7–25.5μ	14–22.1μ	up to 1.5μ	‡3–5(6)μ	——
A. laevis (strain A)	Smith (this paper)	Wound parasite on crayfish	3.5–8.5μ av. 6μ	Same as hyphae	8–11.5μ	20.5–35μ av. 28μ	16–29μ av. 22.5μ	0.5–1μ av. 0.7μ	0.5–1.5μ av. 1μ	Elongate, vermiform, diclinous
A. laevis (strain B)	Smith (this paper)	Wound parasite on crayfish	4–8.5μ av. 6μ	Same as hyphae	8–11.5μ	22.5–29μ av. 26.9μ	19–24μ av. 21.5μ	0.5–1μ av. 0.7μ	0.5–1.5μ av. 1μ	Elongate, vermiform, diclinous
A. astaci	Schikora (12)	Severe parasite of European crayfish	10μ	10μ	13μ	—	—	—	—	——
A. astaci	Rennerfelt (9)	Severe parasite of European crayfish	7.5–9.5μ	Same as hyphae	8.1–9.5μ	41.6–48μ	22.4–28.8μ	"Feinstacheliger, hyaline"	—	Androgynous, not always present

* Peters' (7) conversion. See text. † Coker's (1) conversion. See text. ‡ Material possibly unsuitable. See text.

the oögonia, I believe Coker was referring to oögonia, not to "eggs" or oöspores. Measurements of De Bary's (**2**) plates show that De Bary probably used the French "ligne" (line) equal to 2.256 mm. My reconversion of De Bary's values gives the size of oögonia in *A. laevis* as 26.2–31.3 μ, thus agreeing more closely with Peters' conversion than with Coker's. The thick oöspore walls reported by Peters (**7**) in his form of *A. laevis* parasitic on beets, and likewise by Coker for his typical form, are probably caused by the onset of degeneration in the oöspore as suggested by Drechsler (**3**). I have observed that application of glycerine or lactophenol will cause an oöspore wall to swell, in a few seconds, to several times its normal thickness, thus rendering preserved material unsuitable for measuring. From the above considerations, it may be seen that there are no serious discrepancies between the measurements I have given for my isolates and the values previously reported by several other workers for *A. laevis*. With the identity of my isolates established on morphological grounds, it is of interest to compare their physiological traits with those reported by other workers for *A. astaci* and *A. laevis*.

Since my isolates were obtained from crayfish, a survey was made of the literature on the European crayfish disease (Krebspest), which is caused by *Aphanomyces astaci* (= *magnusi*) Schikora. This disease, which first swept through Europe in the 1860's and 70's, has been discussed at length by Schikora (**11**, **12**), Schäperclaus (**10**), Nybelin (**5**, **6**), Rennerfelt (**9**), and a number of others. Although some of the data are contradictory, the measurements summarized in table I show that *A. astaci* differs from *A. laevis* not only in the size of its hyphae and oögonia, but also in the surface of the oögonial wall, which in *A. astaci* is not smooth but finely prickly. A further point, not in itself conclusive, is that *A. astaci* is restricted to the European crayfish *Potamobius* (= *Astacus*) and does not attack the introduced American species *Cambarus affinis* Say (Schäperclaus, **10**). Thus it is evident that although the present fungus has been found on crayfish, it is not the same fungus that causes the European crayfish disease.

A survey of the host range of *A. laevis* reveals a rather great variation among the types reported by several workers. De Bary

(**2**) described *A. laevis* from Germany as occurring on dead insects in water. Coker (**1**) in the United States finds it typically saprophytic, but describes a variety as parasitic upon desmids and diatoms. In addition, *A. laevis* has long been considered as contributing to the root rot (Wurzelbrand) of sugar beets in Europe (Peters, **7**), although this identification has been questioned by Drechsler (**3**). Finally, my isolates have been obtained from crayfish, but have been found to grow very well saprophytically on plant or animal material. In view of Peters' (**7**) findings, I have made several attempts to infect pea and beet seedlings with my isolates, but to date have observed no damping-off. However, as Peters (**8**) reports that *A. laevis* is responsible for only about 11 per cent of the observed cases of root-rot of beets, it cannot be considered a particularly active parasite, and my failure to obtain infection is neither surprising nor significant. From the above considerations it is apparent that the several physiological descriptions of *A. laevis* are not in agreement. This disagreement may well be the reflection of a real variability in nature, a variability strikingly shown in the reproductive habits of the two morphologically similar strains of *A. laevis* described in this paper.

The genus *Aphanomyces* is strongly inclined to be parasitic upon animals (Gicklhorn, **4**) as well as on the higher plants (Peters, **7**; Drechsler, **3**) and the lower plants (Coker, **1**), while generally able to exist saprophytically in culture or on plant or animal remains. The fungus which has been dealt with in this paper exemplifies well the mild facultative parasitism which may in other forms have led to true parasitism. For reasons stated earlier, the present isolates are considered merely wound parasites whose mild nature makes it unlikely that they can be dangerous pests except where crayfish are kept under crowded and unhealthful conditions.

SUMMARY

1. *Aphanomyces laevis* De Bary has been found occurring as a mild, facultative parasite in the wounds of young crayfish (*Cambarus Clarkii*) kept in the laboratory.

2. Two morphologically similar strains are described, one of which produces few zoöspores and many oögonia, the other of which forms few oögonia but many zoöspores.

3. In identifying these strains as *Aphanomyces laevis*, the physiological variability of the species as reported by previous authors is considered. In view of this variability, the differences in the reproductive habits of the two morphologically similar isolates discussed in this paper are of interest.

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CONTRIBUTIONS TO THE LIFE HISTORY OF A SYSTEMIC FUNGOUS PARASITE, CRYPTOMYCINA PTERIDIS [1]

SARA BACHE-WIIG [2]

(WITH 26 FIGURES)

A disease of bracken [3] (brake) caused by *Cryptomycina Pteridis* (Rebentisch ex Fries) von Höhnel is wide-spread in America as well as in Europe and occurs also in northern Asia. The disease has been called "leaf roll" (Killian, **1918**) and is characterized by curling and stiffness of the pinnules of young fronds, accompanied by yellowish-green discoloration and the appearance of brown spots and of abundant black stromatic areas on the lower surface of the pinnules between the veinlets (FIGS. 2, 3). These areas resemble the sori of an *Asplenium*, as observed by Rebentisch (**1804**). Formation of conidial fructifications during the

[1] A thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer gratefully acknowledges the valuable advice and criticism received from Professor H. M. Fitzpatrick during the progress under his direction of these studies.

She is also indebted to Professor H. H. Whetzel for encouragement and for aid in securing materials, to Professor A. J. Eames for help in interpreting the structure of the bracken, to Professor L. Knudson for directions for growing bracken in culture, and to Professor L. M. Massey and Professor Frances Grace Smith for critical reading of the manuscript.

[3] Since the bracken, which, under the name *Pteridium aquilinum* (L.) Kuhn (= *Pteris aquilina* L.), has been considered the most cosmopolitan of ferns (Christ, **1910**), is now treated as several species, it should be made clear that the species used in the observations and experiments reported on in this paper is the eastern bracken, the *Pteridium latiusculum* (Desv.) Hieron. ex R. E. Fries of Broun's "Index to North American Ferns" (**1938**), while the Eurasian species, host of the fungus studied by Killian (**1918**), is, according to the same authority, *Pteridium aquilinum* (L.) Kuhn, represented in America by the variety *P. aquilinum lanuginosum* (Boug.) Fernald of western North America, the Great Lake region and eastern and southern Quebec. A third North American species is *Pteridium caudatum* (L.) Maxon., found from Florida to Louisiana and southward.

growing season is followed by the development of stromata in which asci mature the following spring.

Photographs of diseased and healthy fronds and pinnules, and a series of excellent drawings of the development of acervuli and ascus fructifications [4] illustrate the account which Killian (**1918**) gives of the disease. Mains (**1935**) gives a good photograph of overwintered, mature ascus fructifications.

Further consideration of the disease will be prefaced by (1) a brief description of the morphology of the host, and (2) a consideration of the fruiting structures of the parasite.

THE HOST. MORPHOLOGY

The bracken has a stout, black, creeping underground stem rounded above and below, with a narrow ridge along each side. "The young plant starts as a single axis bearing 7 to 9 alternating leaves, spirally arranged, after which it undergoes distal and equal dichotomy. . . . The two branches burrow downwards into the soil, bearing leaves alternately right and left; in the later phases of their development they also show dichotomies, but with unequal shanks: . . ." (Bower, **1923**).

The rhizome tips and the very young leaves, which will hereafter be called stem buds and frond buds, respectively, are lighter in color than the mature rhizome. They can be distinguished from each other by their orientation and form: the stem buds are straight and usually horizontal, while the frond buds are vertical, and bent or coiled at the tip.

The fronds of the bracken grow singly from the rhizome. The first coiled young fronds or crosiers appear above ground in May, unrolling and maturing progressively from base to tip, in the manner characteristic of ferns.

Cross sections of the rhizome show that a band of cortical sclerenchyma forms a hard outer rind, broken only by the two ridges

[4] The author has found no report of the occurrence of the perfect stage of *Cryptomycina Pteridis* on any hosts other than species of bracken. The imperfect stage, however, under the name *Fusidium Pteridis,* has been reported on three other ferns: in Europe on *Phegopteris* by von Thümen and by Voss (Lindau, **1907**), and on *Aspidium spinulosum* by von Thümen (Lindau, **1907**); and in the United States on *Aspidium marginale* by Trelease (**1884**).

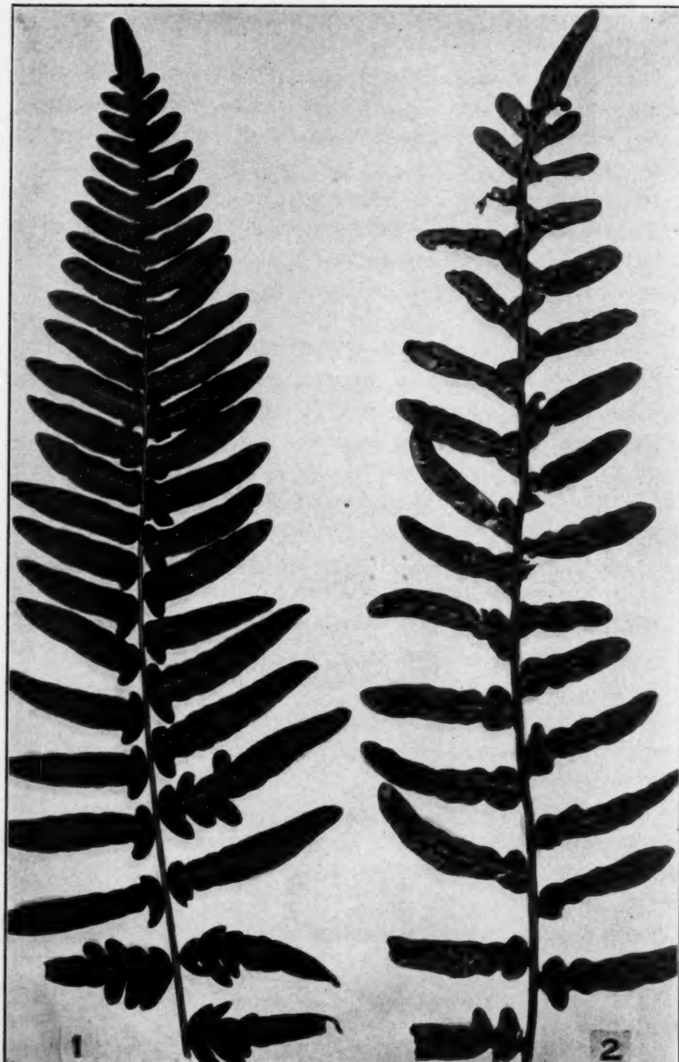


FIG. 1, pinna of healthy bracken; 2, pinna of diseased bracken, systemically infected with *Cryptomycina Pteridis*. Figs. reduced ⅐. (Photograph by Mr. W. R. Fisher.)

already mentioned, in which ". . . the sclerenchyma is replaced by highly ventilated parenchyma . . ." (Bower, **1923**). Strips of sclerenchyma are present also near the center of the rhizome, where they separate an inner series of vascular strands from an outer series, both imbedded in parenchyma. Each strand is made up of an encircling endodermis, very conspicuous because of the browning of its cell walls, below which lies pericycle, followed by sieve tubes and next by thick-walled tracheids and vessels which occupy the center of the strand. Parenchyma cells are found in both the xylem and the phloem (Bower, **1923**).

A median longitudinal section of a stem bud (FIG. 8) shows that the rhizome tip is slightly concave, and that the depressed embryonic region is protected by hairs. At the bottom of the depression lies the large apical cell. The cells adjacent to the apical cell are also large, but farther away, as a result of repeated cell division, they are much smaller. Differentiation of tissues takes place a very short distance behind the meristematic tip.

A stipe (petiole) in cross section differs in outline from a rhizome, being flattened to concave on the anterior side, except at the base. Its tissues, including ventilating tissue, are essentially the same as those of the rhizome. Although the frond receives vascular strands from both the inner and outer series of the rhizome (Bower, **1928**), their arrangement is not maintained in the stipe, and the sclerenchyma, instead of forming a broken band, has the form of a T or a Y.

A median longitudinal section of a frond bud shows the developing lamina (blade). In very young buds this is a simple curved process of undifferentiated cells; in older buds, pinnae are visible as lobes of the process.

THE PARASITE. FRUITING STRUCTURES AND TAXONOMIC POSITION

The perfect stage of the fungus was first seen by Fuckel (**1869**), who describes the asci as sessile, 8-spored, $64 \times 14\ \mu$, and the ascospores as subdistichate, perfectly oval, one-celled,[5] mostly biguttulate, hyaline, $8 \times 6\ \mu$. A more complete description is given

[5] The description of the ascospores as elongated and triseptate, given by Cooke (**1871**), is obviously based on the examination of the asci of some other fungus occurring in company with *C. Pteridis.*

by Starbäck (**1889**), who observed that the ascospores might be distichate, transversely monostichate, or crowded in the upper part of the ascus, and that while most of the spores are ovoid, some are ellipsoidal and some even round, measuring $8.5–12\ \mu \times 5–6.5\ \mu$, most often $10.5 \times 6\ \mu$, the globose ones $7.5\ \mu$. This variation in arrangement, form, and size of ascospores agrees entirely with observations on material collected at Ashfield, Mass., in June, 1939, by the present investigator. Absence of paraphyses is mentioned by several authors (Saccardo, **1883**; Karsten, **1885**; Starbäck, **1889**), while Rehm (**1896**) first records their presence and describes them as filiform, very sparse, and delicate. Filaments of this description are present in the materal from Ashfield but since they evidently are remains of an interthecial stroma they are not true paraphyses but paraphysoids, in the sense in which the terms are used by Gäumann and Dodge (**1928**).

The asci are borne in a flat layer in apothecium-like, subepidermal fructifications having an upper covering as well as a basal part, both characterized by vertically arranged cells with dark, thickened walls (Killian, **1918**). As emphasized by Nannfeldt (**1932**), the investigations of Killian prove the purely stromatic origin of the plectenchyma which encloses the ascus layer, since it is formed from an originally compact tissue, the cells of which become loosened and absorbed as the asci develop. This fruit body, which lacks a true apothecial envelope as defined by Nannfeldt (**1932**), will be referred to in this paper as a "pseudothecium," following the usage of Nannfeldt. When dead infested fronds overwintered on the ground become soaked by spring rains, the covering of the pseudothecium becomes irregularly torn, exposing the asci from which spores begin to be shed at about the time the first bracken fronds are unrolling. For several weeks ascospores continue to be freed after rains (Killian, **1918**).

Recognizing the dothideoid structure of the pseudothecium of this fungus, Fuckel (**1869**) placed it in the genus *Phyllachora*[6] Nitschke, of the Dothideaceae Nitsch. It was transferred by Rehm (**1896**) to the Phacidiaceae and placed in the genus *Cryptomyces* erected by Greville (**1826**) on the willow parasite *Crypto-*

[6] For the synonymy of the perfect stage of the fungus see Oudemans (**1919**).

myces maximus (Fries) Rehm, which forms massive sub-peridermal ascus fructifications on the branches of its host, becoming erumpent at maturity and exposing the flat ascus layer by irregular dehiscence of its covering. Von Höhnel (**1917**) removed the bracken parasite from the genus *Cryptomyces* Grev. and made it the type of a new genus, *Cryptomycina* v. Höhn., because its fruit body is sub-epidermal instead of sub-peridermal. And since the position of the fruit body in relation to the substratum is the basic character for von Höhnel's delimitation of families within the Phacidiales, the two genera are placed in different families, *Cryptomyces* in the Cryptomycetaceae and *Cryptomycina* in the Phacidiaceae. (The statement by Gäumann and Dodge (**1928**) that the bracken parasite belongs to the Cryptomycetaceae of von Höhnel's treatment is based upon the assumption that the fungus conforms to the genus *Cryptomyces* Grev. as limited by von Höhnel, whereas he expressly makes this fungus the type of the genus *Cryptomycina* v. Höhn.) Whatever criticisms may be leveled at von Höhnel's treatment of the Phacidiales (*e.g.* Petrak, **1924**; Nannfeldt, **1932**) his separation of *Cryptomycina* v. Höhn. from *Cryptomyces* Grev. is upheld by the recent investigations of Nannfeldt (**1932**) who shows that the massive fruit body of *Cryptomyces maximus* has a distinctive four-layered structure. A basal layer of loosely woven, hyaline hyphae is followed by a wider, more compact layer showing thick, faintly colored walls, while a third, narrow layer is made up of cells with very thick, dark walls, forming a compact, sharply delimited tissue toward the outside where it gives place to the fourth layer, a compact palisade-like tissue of straight, parallel, perpendicular, hyaline, thin-walled septate hyphae. The asci develop in this outer layer. The more delicate fruit body of *Cryptomycina Pteridis* has a different, far more simple structure. As described by Killian (**1918**), it consists of a compact plectenchyma of isodiametric cells with thick, brown walls, surrounding a central layer of hyaline, thin-walled, perpendicularly elongated cells. The asci develop in the central layer. In the light of the two investigations just cited, Nannfeldt (**1932**) considers both genera to have purely stromatic fructifications (pseudothecia), and he therefore places them not in the

Phacidiaceae of his Ascohymeniales, but in the Pseudosphaeriales of his Ascoloculares.

The conidia of *Cryptomycina Pteridis* were first described as *Fusidium Pteridis* by Kalchbrenner (**1861**), who considered them to be those of another fungus growing in company with *C. Pteridis* (called by Kalchbrenner *Dothidea Pteridis* Fries). As described by him the conidia are cylindrical, straight or slightly curved, blunt at both ends, hyaline. Six years earlier, however, Strauss (**1855**) had published a general description of the development of *Dothidea Pteridis* including observations on the formation of the conidial fructifications, described as vesicles with milky contents of "... unzählige weisse walzige Körperchen (Spermatien)." The description was not amplified, and Fuckel (**1869**) is credited with being the first to consider that these spores represent the conidial stage of *C. Pteridis*, called by him *Phyllachora Pteridis* (Rebent.) Fuckel. Killian (**1918**) has conclusively proved that the asci and the conidia belong to the same fungus. In addition to showing the successive development and basic structural similarity of the two kinds of fructifications, he has described cases in which one half was producing conidia while the other half of the same structure was developing into an ascus fructification. This has been observed also by the present investigator in material from Ashfield, Mass.

It is interesting that the first recorded measurements of the conidia were not made in Europe but in America, by Peck (**1875**), who gives the length of the conidia as .0004–.0005 inch. Expressed as 10–12 microns these measurements are quoted by Karsten (**1885**), and he, in turn, is quoted by Rehm (**1896**). Later investigators have found that both larger and smaller conidia occur. Bubák and Kabát (**1906**) give the dimensions as 9–13 $\mu \times$ 2.5–4 μ, while von Höhnel (**1925**) gives 9–16 $\mu \times$ 2–4.5 μ. Typical conidia from diseased fronds in the Smith College Plant House ("descendants" of material collected at Ashfield, Mass.), measured at different times by the present investigator, varied within the limits 7–16 $\mu \times$ 3.5–5.5 μ. Material from Ringwood, near Ithaca, N. Y., measured 10–16 $\mu \times$ 4–5 μ. Occasionally a much larger conidium could be found, up to 23 μ in length.

Measurements by several American investigators, including

FIG. 3, pinnules of systemically diseased bracken, × 2, showing intercostal stromata of *Cryptomycina Pteridis;* 4, tip of younger diseased pinna, × 8, with whitish droplets of conidial ooze on dark areas; 5, germinating conidium in dark-field illumination, × 510. (Photographs by Mr. W. R. Fisher.)

Davis (**1924**) and Gilman and Archer (**1929**), have been purposely left out, because the present writer is not yet ready to accept either the extensive synonymy of Gilman and Archer (**1929**) or a more limited one such as that of Davis (**1924**).[7]

As noted by Kalchbrenner (**1861**), Peck (**1875**), and others, the conidia are held together in a sticky mass as they are forced out of the conidial fructifications, and may form little whitish to amber globules on the surface of the dark spots (FIG. 4). Sometimes they are forced out as cirrhi. It is evident that the conidia are not borne by air currents but by water.

The fructifications from which these conidial masses ooze have been described by Killian (**1918**) as follows. At first they are small structures with a loose basal part and a flat layer of conidiophores. By gradual development the acervulus increases in size laterally, and usually also deepens, taking on a more spherical form. The basal part becomes compact, and its cell walls thick and brown. A covering of similar cells is absent or weakly developed except in winter acervuli (or pycnidia) in which it is typical. Adjacent fructifications may coalesce. From the conidiophores conidia are successively abjointed apically and to some extent from lateral branches. Conidial production continues during the entire vegetative season and even into the winter.

Whether this conidial stage should be referred to the genus *Gloeosporium* in accordance with Bubák and Kabát (**1906**), or to the genus *Cylindrosporium* in accordance with Gilman and Archer (**1929**), or to some other genus,[8] is a queston which lies outside the province of this paper. But as to identity, the conidial material from Massachusetts used in the experiments described in this paper clearly conforms to the description of *Gloeosporium Pteridis* (Rebent.) Bubák and Kabát in the form, size, and aseptate character of the conidia (Bubák and Kabát, **1906**), while the fructifica-

[7] For the synonymy of the imperfect stage, see also Bubák (**1916**), and Seymour (**1929**).

[8] Von Höhnel (**1925**) makes the imperfect stage of this fungus the type of a new genus of the Fungi Imperfecti, *Cryptomycella Pteridis* (Kalchbr.) v. Höhn., emphasizing that ". . . der Pilz ein gut entwickeltes braunes Stroma besitzt und neben halb offenen auch geschlossene Fruchtkörper hat. . . ." He also records the presence of a second type of conidium characterized by pointed ends and small dimensions ($6–8\,\mu \times 1.5\,\mu$).

tions from which they arise are just like those described and figured by Killian (**1918**). That this American form is the same as the European one was further confirmed by the similarity in form, size, and manner of germination of conidia of *Cryptomycina Pteridis* collected in Massachusetts on the eastern bracken and of conidia of *Cryptomycina Pteridis* collected on the bracken of Europe near Stavanger, Norway.

THE DISEASE. REVIEW OF PREVIOUS INVESTIGATIONS

The disease and its causal fungus have been studied in detail by Killian (**1918**) only. His full and well illustrated account is based on field observations made from May to November, correlated with studies of fixed, sectioned and stained material of host and parasite, beginning with the young diseased fronds and carried through to the formation of ascospores on dead fronds the following spring. Although Killian has presented a complete picture of the life history of the parasite (to which frequent reference has been made in discussing the fruit bodies of the fungus), the present paper will consider in detail only those portions of Killian's investigations which deal with the relation of host and parasite—inoculation, incubation, infection, and appearance and distribution of the fungus hyphae in the tissues of the fern frond—, since these alone have a bearing on the studies presented in the present paper. The following, then, is a synopsis of Killian's account as it bears on the relation between *Cryptomycina Pteridis* and its host:

> Inoculation with ascospores occurs after rain, beginning about the middle of May and extending into June. Inoculation with conidia takes place from June into November. The ejected ascospores fall upon the surface of the wet fronds and, as the water drops evaporate, are drawn with the last traces of water by capillarity through the stomata of the lower epidermis into the substomatal chambers where they germinate.
>
> (These conclusions in regard to inoculation were based on the study of sections of young, naturally infected fronds, in which structures interpreted as germinating spores were observed and figured by Killian. His attempts to germinate ascospores in drops of water on living pinnules of bracken in

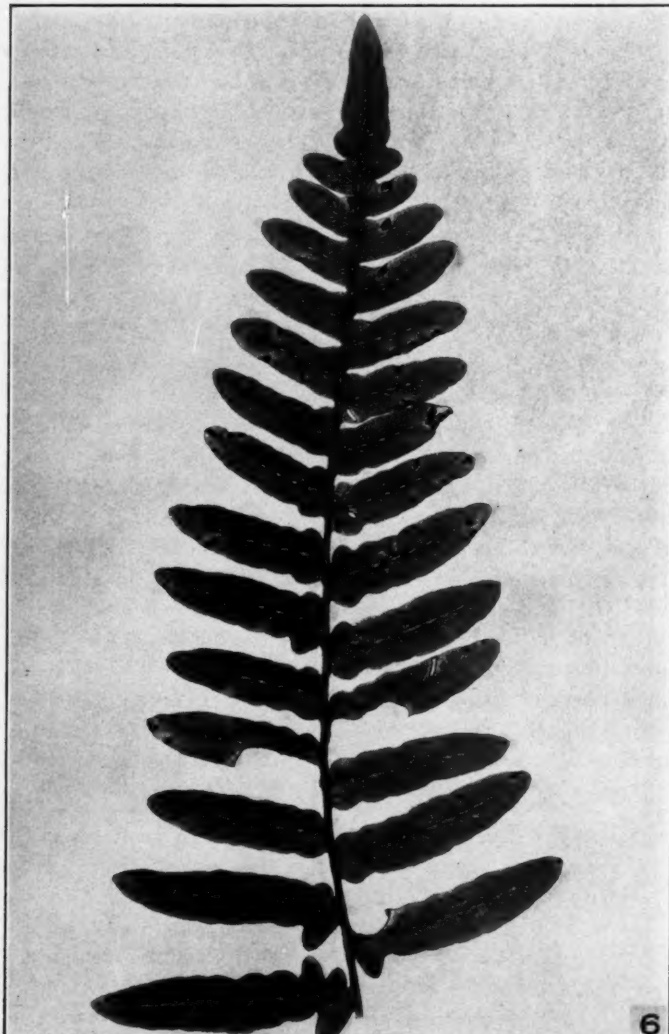


FIG. 6, pinna of diseased bracken from Ringwood, N. Y., × 1⅓, showing scattered localized lesions, many and small on older parts, few and large on younger parts—lesions similar in character and distribution to those on artificially inoculated fronds. (Photograph by Mr. W. R. Fisher.)

gave extremely variable results, while attempts to germinate ascopsores in drops of water on living pinnules of bracken in moist chambers failed completely, as did also all attempts to germinate conidia. Germination of ascospores and growth of an ascomycete were obtained on a solid medium from spores ejected from bits of dead, overwintered, infested fronds—but the fungus which developed was not *C. Pteridis*. It proved to be a saprophyte. Successful infection experiments in the natural habitat are mentioned but not described.)

Only young fronds which have just unrolled are attacked. Older fronds are immune, their immunity extending to the still embryonic tip.

In the beginning the hyphae are always intercellular. They grow in particular abundance in the substomatal chambers and adjacent intercellular spaces.

The incubation period varies in length. It is usually two days but may be six. (The maximum length of the period is not given. Killian regards the incubation period as the period elapsing between a spring or early summer rain sufficient to bring about soaking of dead infested fronds and subsequent ejection of ascospores, and the appearance of the first symptoms of disease in young fronds exposed to a shower of these spores.)

The first symptom of infection is the boat-shaped arching of the pinnules, making the lower surface concave. Later the foliage takes on a yellowish-green color, especially on the lower surface, contrasting with the blue-green color of healthy fronds. The yellowing involves the tissue between the veins, the veins themselves remaining normal in color. Symptoms characteristic of the further progress of the disease are the strong inrolling of pinnule tips and the upward lift of the pinnules. Brown dots, signalling the formation of conidial fructifications, appear in the yellow areas, followed by black dots which mark the initiation of ascus fructifications. The black spots grow and become confluent, finally occupying the intercostal areas as black stromata.

It is typical of most diseased fronds that the series of symptoms and signs described above appears first on the basal pin-

nae and progressively on the upper pinnae as these develop, until the whole frond is involved. Some fronds, however, show a lighter infection, with general symptoms of a less conspicuous nature. "Es ist nun bemerkenswert, dass hier die ältesten schwarzen Fruchkörper nicht wie sonst, regelmässig auf die ältesten Blattfiedern verteilt sind, sondern unregelmässig zerstreut vorkommen."

The explanation given of this difference in type of symptoms is a difference in resistance of the fronds. In a susceptible frond the fungus is assumed to spread rapidly through the tissues, primary infection becoming unidentifiable through the secondary vegetative spread of the fungus or by additional infections due to conidia. (Rapidly growing fronds may present a partial infection of this type in which the edges of the pinnules remain entirely unaffected.) In a resistant frond, on the other hand, it is assumed that the fungus meets with such opposition that its growth is limited ". . . mehr auf seine Ursprungsstellen, da eben, wo zufällig die Ascusspore hingeschleudert wurde," and the places of primary infection ". . . sind naturgemäss nach den Gesetzen des Zufalls zerstreut."

The development of typical symptoms is correlated with the activities of the fungus in the tissues of the host. Spreading out, according to Killian's interpretation, from the substomatal chambers, at first intercellular and forming a loose weft, the fungus later sends compact side branches into the host cells, attacking the nucleus and the chloroplasts, and finally reducing the cell contents to a formless lump. The hyphae within the cells show stronger growth than the intercellular mycelium and become much branched, filling the invaded cell. This invasion takes place near the infection courts, while farther away the hyphae are always intercellular.

In the substomatal chambers and adjacent regions, where the fungus grows most freely, are initiated the conidial fructifications.

The veins are not invaded by the fungus.

The hyphae are characterized by very thin walls, thickly granular cytoplasm, many vacuoles, and nuclei with variable

affinity for stains, in some cases staining intensely, in other cases faintly.

INVESTIGATIONS

PART I. STUDIES ON THE SYSTEMIC NATURE OF THE DISEASE

In the course of field observations beginning in 1926, the present investigator has been struck by certain aspects of the disease which indicate systemic infection.[9] The assumption that typically diseased fronds were infected with the mycelium of the parasite from the time of their initiation as buds was based on the following observations:

1. The characteristic abundance and even distribution of the stromata.
2. The appearance of symptoms first on the oldest (basal) pinnae and then progressively on younger pinnae.
3. The fact that when the lowest pinnae of a frond were healthy, the tip of the frond was also healthy, in spite of the well-known fern character of possessing a still embryonic tip while the lower pinnae are maturing. (These three observations were also made by Killian. He explained the first and second phenomena as due to the rapid spread of the invading fungus from the points of infection. He explained the third by assuming the development in the maturing portion of a frond of a resistance that extends to the younger parts.)
4. The slow spread of the disease. It was seen year after year in the same patches of bracken while neighboring ones remained healthy.

These were indications only. Evidence was sought along two lines of investigation: (1) Buds of rhizomes that had produced typically diseased fronds were examined microscopically in order to determine whether mycelium was present in their tissues; and

[9] Although the theory that in the leaf roll disease of bracken systemic infection is present has been expressed to the writer independently by more than one mycologist during the progress of these investigations, only two published statements that reflect such a view were found, namely those of Rostrup (**1885**) and Lind (**1913**), the second being based upon the first.

(2) rhizomes which had borne typically diseased fronds were transplanted into an environment where spore inoculation would be excluded, in order to determine whether new fronds produced under such conditions would also be diseased.

A. Systemic Infection of Bracken Buds

Buds from bracken rhizomes bearing diseased fronds, and buds from rhizomes bearing healthy fronds were cut in pieces and fixed. Material from both sets of rhizomes was gathered in Ashfield, Mass., in late fall, and at Ringwood, near Ithaca, N. Y., in midsummer. Macroscopically, the buds from the two sets of rhizomes looked alike. Some of the buds were cut free-hand, others were imbedded in paraffin (52–54°) and cut into sections 5 to 17 μ thick. The stains chiefly used were thionin and orange G according to the method of Stoughton (**1930**), and Heidenhain's haematoxylin with light green. *A fungus was found in all buds from rhizomes bearing diseased fronds, whereas no fungus was found in any of the buds from rhizomes bearing healthy fronds.*

In order to determine the distribution and characteristics of the mycelium in the invaded buds, an intensive study was made of sections of one stem bud and one frond bud. For purposes of comparison, sections showing the mycelium of *Cryptomycina Pteridis* in typically diseased uncurled fronds of bracken were studied. In sections of both kinds of buds the distribution of the fungus in the tissues of the bracken was very patchy, neither evenly distributed nor concentrated in any particular tissue.

In the stem bud, the fungus was found in the following tissues: (1) Undifferentiated tissue of the tip, only 11 cells removed from the apical cell, (2) sclerenchyma (both that of the subepidermal sheath and that of the central strands), (3) cortical parenchyma, (4) endodermis, (5) pericycle, (6) phloem, (7) xylem. In the frond bud also, the fungus was present in cortical and in vascular tissues, and was seen also in the epidermis.

The fungus is both inter- and intracellular (FIGS. 9, 12–15). Near the tip of the stem bud, the cells of invaded areas are usually alive and normal in appearance even when the fungus is in contact with the nucleus,[10] which is often the case (FIGS. 10, 11). The

[10] Later study of rapidly growing buds showed that the nucleus of an invaded cell may undergo mitosis (FIG. 16).

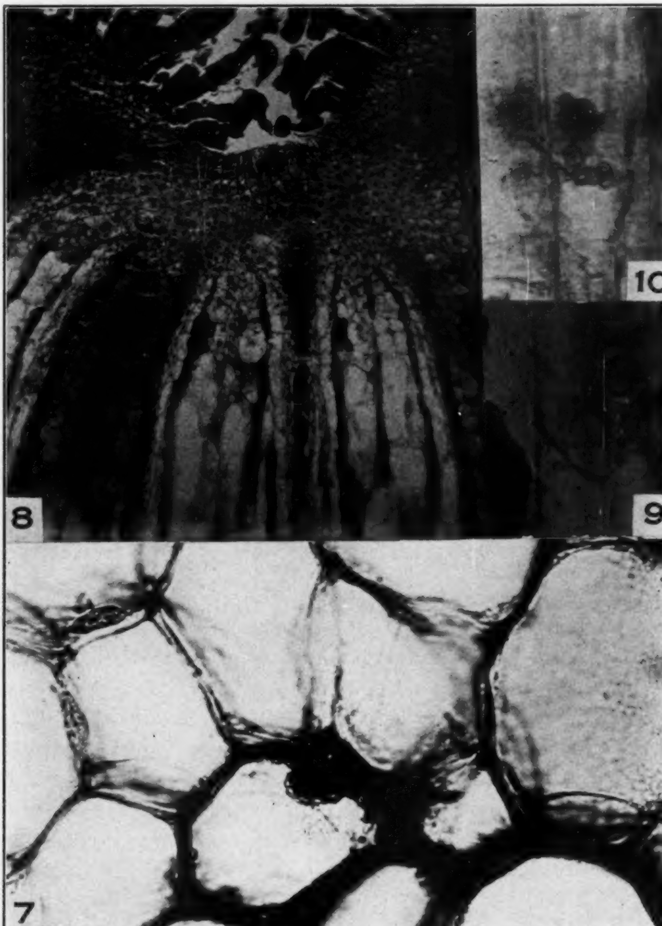


FIG. 7, cortex of systemically diseased bracken bud, × 500, showing dead cell with thickened, discolored walls and cluster of dead fungus hyphae; 8, portion of tip of bracken stem bud (diseased), × 92, long. section, showing large cells adjacent to apical cell (apical cell not included in section), undifferentiated tissue behind apical region, and differentiated tissues farther back; 9, intracellular mycelium in pericycle of diseased stem bud, × 631, long. section; 10, hyphae in contact with nucleus of pericycle cell, × 732, long. section. (Fig. 7 from photograph by Mr. W. R. Fisher; 8–10 from photographs made with the help of Professor Helen A. Choate.)

fungus hyphae are stout, and the intracellular mycelium is characterized by its prolific branching and its tendency to fill the entire cell (FIG. 13), as noted by Killian (**1918**) for intracellular mycelium of *Cryptomycina Pteridis* in frond chlorenchyma. It is easier to see the fungus in the tip of the bud than in portions a few millimeters behind the tip, since in the older parts the mycelium together with the walls of the invaded cells tends to take on a dark color. The invaded regions, however, are easily picked out because of this discoloration. These regions remain limited in extent, and in them the cells of both host and parasite appear to be dead (FIG. 7).

The tip of the frond bud with its invading fungus presents a picture similar to that presented by the tip of the infected stem bud.

When stained with thionin and orange G, the mycelium in the bud tips took on a bright yellow color, limited to the peripheral region of the hyphae, which made it easy to pick out areas of fungus invasion under the low power of the microscope. The cytoplasm of the hyphae stained faint bluish purple, the nuclei bright purple. Behind the stem tip, the bright yellow coloring was replaced by dirty yellow, and still farther back, as already described, the fungus tends to become dark. A brownish color was also characteristic of hyphae of *C. Pteridis* found in the vascular tissue of the uncurled frond. Some millimeters behind the frond tip, the yellow stain became lighter, while the cytoplasm took on a strong purplish color. The hyphae of *C. Pteridis* in the intercellular spaces of chlorenchyma in the uncurled frond varied greatly in diameter, thick and distinctly club-shaped branches being discernible here and there. The mycelium of these intercellular spaces had the thin walls and brightly-staining cytoplasm described by Killian (**1918**).

B. Production of Diseased Fronds by Transplanted Rhizomes which had Previously Borne Diseased Fronds

In September, 1931, pieces of bracken rhizomes which had borne diseased fronds and pieces of rhizomes which had borne healthy fronds in Arnot Forest, Schuyler County, N. Y., were washed and transplanted to flats, wintered out-of-doors in Ithaca, N. Y., remote from any source of natural inoculation, and brought into the greenhouse in April and May. In the greenhouse, fronds appeared at irregular intervals from the beginning of May.

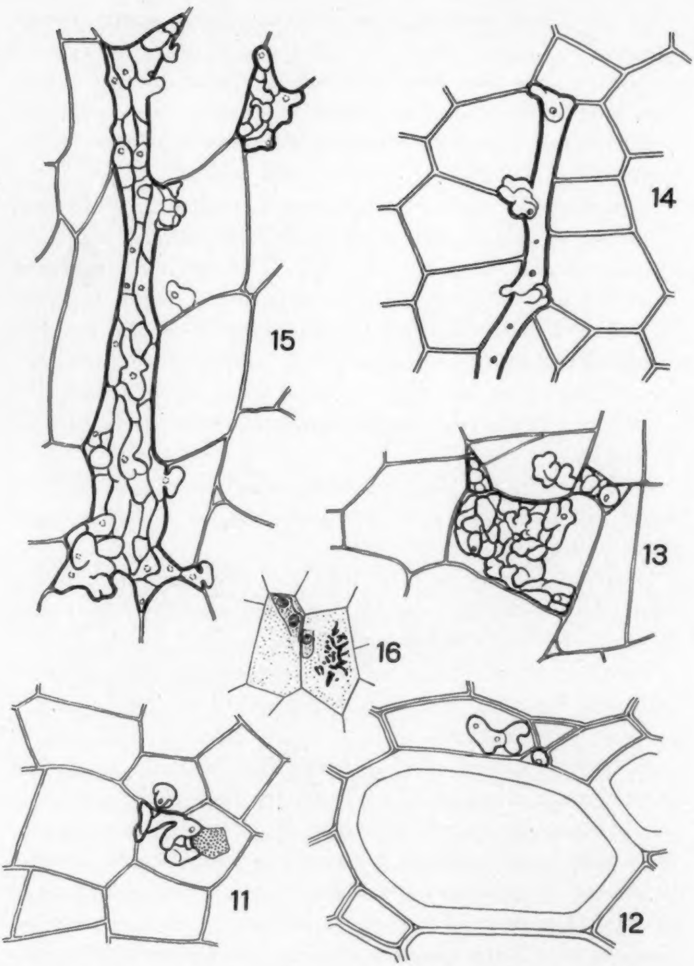


FIG. 11, inter- and intracellular mycelium of *Cryptomycina Pteridis* in young cortical tissue, long. section; 12, mycelium in xylem, cross section; 13, 14, mycelium in young cortical tissue, long. sections; 15, intercellular mycelium and haustorial branches in young phloem, long. section; 16, intracellular hyphae in undifferentiated tissue near apical cell, one of invaded cells in early anaphase, long. section. All figures about × 700. Thickness of walls of host cells somewhat exaggerated in figures 11, 13, 14, and 15.

All the fronds from rhizomes which had borne healthy fronds were healthy.

Of the fronds from rhizomes which had borne diseased fronds, some were healthy, but others showed typical symptoms of the disease when their pinnae unrolled, and later their lower surfaces became covered with the characteristic black stromata.

A second experiment of the same sort was run at Smith College, Northampton, Mass., with rhizomes dug in Ashfield, Mass., on October 16 and November 21, 1935. The flats were examined from time to time, and notes taken on the fronds which appeared. By July, 1936, observations were complete for 51 fronds from rhizomes which had borne diseased fronds, and by August 18, 1936, for 31 fronds from rhizomes which had borne healthy fronds.

All the 31 fronds from rhizomes which had borne healthy fronds were healthy.

Of the 51 fronds from rhizomes which had borne diseased fronds, 39 were diseased, 6 were healthy, and 6 showed doubtful traces of disease.

Observations made in 1937, 1938, and 1939 gave the same results: *Healthy rhizomes continued to produce healthy fronds only. Diseased rhizomes continued to produce diseased fronds, but not all fronds produced by diseased rhizomes were necessarily diseased.* And in at least one case, all fronds produced by such a rhizome appeared to be healthy.

In the case of a number of fronds from rhizomes which had borne diseased fronds, a young (rolled) portion of the frond was cut off before any macroscopic symptoms or signs of diseases were discernible, fixed, sectioned and stained in order that the presence or absence of the fungus at this early stage of development could be checked against the presence or absence of disease symptoms observed later in the maturing fronds. The immature fronds varied in height from 1.5 to 14 cm. The wounds were seared. The work was carried on from November, 1935, to June, 1936. The observations made are recorded in table 1.

These data show that *no fungus was found in pieces cut from young fronds which later showed no symptoms of disease, that the fungus was found in pieces cut from fronds which later showed*

typical symptoms of disease, and that no fungus was found in the piece cut from the frond which later showed a few scattered lesions.

TABLE 1

MICROSCOPIC EXAMINATION OF PIECES CUT FROM YOUNG FRONDS CHECKED AGAINST CONDITION OF SAME FRONDS WHEN MATURE

Date of cutting	Result of microscopic examination of pieces cut from young fronds: presence of fungus indicated by + absence of fungus indicated by −	Date of observation	Condition of mature frond: diseased condition = + healthy condition = −
Nov. 21	+	Feb. 12	+
Nov. 21	+	Feb. 21	+
Feb. 19	+	March 10	+
April 1	+	May 3	+
April 1	−	May 3	A few scattered lesions
May 3	+	June 2	+
May 10	+	June 2	+
May 10	+	June 2	+
May 10	+	June 2	+
June 3	−	June 23	−
June 3	−	June 23	−
June 3	−	June 23	−

In order to determine the condition of a rhizome which had previously borne diseased fronds but from which healthy fronds now grew, a stem bud and a frond bud originating near the base of one of these healthy fronds were examined. No fungus was found in either bud.

On the basis of the evidence presented in Part I of this paper it is concluded that the leaf-roll disease of bracken is characterized by systemic infection. *Cryptomycina Pteridis* overwinters in rhizome buds and frond buds where it is present as inter- and intracellular mycelium in scattered areas of limited extent in the various host tissues, including the undifferentiated tissue of the extreme tip. During the growing season, its growth keeps pace with the growth of the stem buds, but the fungus does not spread in the maturing rhizome, remaining confined to limited areas where its cells apparently soon die. In the developing frond, however, its growth is luxuriant, especially in the intercellular spaces.

Killian (**1918**) considers infection by growth of the fungus mycelium within the host tissues in two connections:

1. To explain the very even and complete infection of typically diseased fronds.

2. As a possible cause of the infection of new sprouts from diseased fronds.

It is only one step further to consider the entire infection of such completely infected fronds as due to the growth of mycelium which has overwintered in the bud. This explains the completeness of the infection, the appearance of symptoms progressively on the maturing portions of the frond, and the apparent resistance of the tip when the base of the frond is not affected. It explains also the variability of the "incubation period" observed by Killian (**1918**) and the frequent shortness of this period (2 days), since the lapses of time thus recorded would be without significance if the bracken fronds were diseased before they appeared above ground.

This view involves a new conception of the development of the fungus in the tissues of the maturing frond. In the systemically infected frond the mycelium does not have its initial development as intercellular mycelium in the sub-stomatal chambers, spreading thence throughout the chlorenchyma (Killian, **1918**). On the contrary, it is present as both intercellular and intracellular mycelium in scattered regions throughout the young frond, and spreads from some of these foci, reaching its greatest development in intercellular spaces and sub-stomatal chambers.

Of the fronds grown in the greenhouse from diseased rhizomes many show symptoms of what may be called incomplete systemic infection. The tips of the pinnae show no dark spots, or the upper portion of the frond shows none, or one side of the frond has many lesions and the other side few, or, as was seen in a single case, the lower pinnae show no lesions while the upper pinnae are typically diseased. It is easy to understand these partial infections when the patchy distribution of the fungus in the bud is recalled, for it then becomes clear how effectively the exclusion of the parasite from certain frond parts would follow the localized disturbance, in favor of the host, of the delicate balance between growth of the host and growth of the parasite.

Other fronds show symptoms of what may be called light systemic infection, characterized by the absence or weak development of the typical leaf-roll symptoms, and by few lesions. This type

of infection may be accounted for by a more profound disturbance of the growth balance in the parasite's disfavor.

PART II. GERMINATION AND INOCULATION STUDIES WITH CONIDIA

Among the questions left unanswered by the studies recorded in Part I were these:

1. Are the conidia and ascospores of *Cryptomycina Pteridis* capable of producing infection?
2. If so, are the symptoms of fronds that become diseased as a result of inoculation with conidia and ascospores similar to the symptoms presented by fronds developed from diseased buds?
3. At what stage of development of the bracken plant is systemic infection initiated?

Any attempt to answer these questions had to be based on inoculation experiments. These were confined almost entirely to experiments with conidia, for two reasons:

1. Ascospores may be obtained only in late spring and early summer from dead infested fronds wintered out-of-doors, while freshly produced conidia may be obtained through summer and fall from living diseased fronds in the field, and for several months longer from fronds grown in the greenhouse. Moreover, conidia on bracken fronds dried in the laboratory may retain their viability for over five months.
2. Pycnidia as well as pseudothecia of *Cryptomycina Pteridis* are frequently found on dead, overwintered bracken fronds, and therefore washings from such fronds do not give an unmixed ascospore inoculum, conidia being present.

A. Germination of Conidia

It will be recalled that Killian (**1918**) failed to obtain germination of conidia. In the present experiment their germination was observed on slides kept in moist chambers, in drops of the following sterilized and unsterilized liquids: distilled water, rain water, tap water, filtrate of humus in rain water. Germination was also observed in filtrates of water in which the following plant parts

had been bruised: fronds of the host (bracken), fronds of *Pteris longifolia* and of *Lygodium japonicum*, and leaf and bud of *Fittonia argyroneum*.

It must be emphasized, however, that the germination of conidia was extremely variable, which probably accounts for the negative results obtained by Killian (**1918**). But their germination in drops of filtrate of water in which bracken fronds had been bruised, although ranging from very poor to excellent, did not once fail completely, as did germination of conidia in water alone. It therefore seems probable that the presence of substances from the bruised frond of the host stimulates the germination of the conidia of the parasite.

Drops of the suspension of conidia used as inoculum in the experiments described in the next section were usually tested for germination. This varied from none to excellent, but it was found that germination on the slide was no criterion of germination on the host as indicated by infection, for a suspension in which not a single germinating conidium was seen on the slide proved just as effective inoculum as a suspension which showed excellent germination.

In germination, a germ tube is produced from either the end or the side of the conidium (FIGS. 5, 17–20). The production of two germ tubes by a conidium was rarely observed. The germ tube usually remains of limited length and unbranched, but sometimes a branch forms at its base.

B. Inoculation Experiments

1. Inoculation of Fronds of Mature Plants

These experiments were carried out on young bracken fronds grown in flats in the Smith College Plant House from healthy rhizomes dug in Ashfield, Mass. Conidia for the inoculum came from diseased fronds grown in the same greenhouses. By means of an atomizer, the fronds were first sprayed with water and then with a heavy suspension of conidia in water. A moist atmosphere was insured by covering the sprayed fronds, together with an adjacent open container of water, with an inverted battery jar for 48 hours.

TABLE 2

INOCULATION OF YOUNG FRONDS WITH CONIDIA

Date	Inoculum		Inoculated fronds		Control fronds	
	Age	Medium of suspension	Number used	Number becoming infected	Number used	Number becoming infected
Aug. 18, 1936	Fresh	Sterile dist. water	3	3	None	
Feb. 6, 1937	125 days	Tap water	1	1	None	
Feb. 21, 1937	140 days	Sterile dist. water	2	1	1	None
March 12, 1937	159 days	Sterile tap water	3	2	2	1?
April 10, 1937	Fresh	Sterile dist. water	7	7	3	None
June 19, 1937	Fresh	Sterile dist. water	3	None	2	None

As will be seen from table 2, inoculation was followed by infection in all experiments except that of June 19, 1937. In this experiment the battery jars were temporarily removed after only 22 hours, and the results of the experiment were not checked until nearly three months from the time of inoculation, because of the unavoidable absence of the experimenter. In the two cases in which one of the fronds inoculated is recorded as not becoming infected (experiments of Feb. 21 and March 12), the frond was in the crosier stage.

While in the two preliminary experiments no controls were used, in the four subsequent experiments they were. The only possible case of infection of a control (experiment of March 12) is that of a single small brown spot on one of the lower pinnae of a frond which may have been in contact with an inoculated frond. When such contact was excluded, no controls showed any symptoms of disease.

The symptoms and signs of the disease on artificially inoculated fronds were: (1) dark leaf spots of various sizes, (2) dark, swollen, distorted areas involving varying amounts of blade, rachis, and stipe tissue, both types of lesions showing lighter areas around the dark regions, and (3) agglutinated masses of conidia on the surface of the spots and distorted areas.

In order to make a careful study of the character and distribution of infection areas on fronds artificially inoculated, the inoculation experiment of April 10, the general results of which are recorded in table 2, was carried out on seven fronds in different

stages of development. A record was made of the height and the extent of expansion of each frond at the time of inoculation, and the distribution and nature of the infection areas that had appeared on each frond were recorded three weeks later.

TABLE 3

CORRELATION OF STAGE OF DEVELOPMENT OF ARTIFICIALLY INOCULATED FRONDS WITH NATURE AND DISTRIBUTION OF INFECTION AREAS DEVELOPED AS A RESULT OF INOCULATION

Stage of development of fronds at time of inoculation, April 10, 1937		Nature and distribution of infection areas developed as recorded on May 1, 1937	
Height of frond in centimeters	Number of pairs of expanded pinnae	Pairs of pinnae showing infection	Pairs of pinnae having largest number of lesions
1.5	0	1st an d2d*	
12.	0	1st and 2d	
13.	1	1st–4th	1st and 2d
10.	3	3d –8th	4th–6th
13.	6	5th–10th	6th and 7th
22.	6	5th–8th	6th and 7th
13.	8	6th–8th	

* And rachis opposite 5th pr. of pinnae.

These records are summarized in table 3. It was also noted that when lesions characterized by large size and by distortion were present, they were limited to pinnae unexpanded at the time of inoculation.

From this experiment are drawn the following conclusions:

(1) Only young fronds and young portions of older fronds of bracken are susceptible to infection by conidia.

(2) The " resistance " of the mature portions of a frond does not impart resistance to the immature portions of the same frond, contrary to the supposition of Killian (**1918**).

(3) The heaviest infection (as measured by the number of infected areas) is found on pinnae which have just expanded or are in the act of expanding at the time of inoculation.

(4) The greatest susceptibility (as measured by the size and degree of distortion of the infected areas) is found in the unexpanded pinnae of a frond. But the number of lesions on these pinnae is small, obviously because they were pro-

tected by their inrolled position, and for the same reason the tip may be entirely free from lesions.

Artificially inoculated fronds never showed the inrolled edges and arched pinnules characteristic of young diseased fronds in the field, nor the equally typical later development of evenly distributed, intercostal stromata (FIG. 2). Since these symptoms and signs were lacking, and since Part I of this paper has shown them to be invariably associated with systemic infection of the host, *it is concluded that such symptoms are characteristic only of diseased fronds which arise from systemically infected bracken plants.*

On the other hand, the symptoms and signs characteristic of artificially inoculated fronds were somewhat similar to those described by Killian (**1918**) on certain lightly infected fronds observed in the field, of which he says: "Solche wenig befallenen Farnpflanzen dokumentieren sich auch dadurch, dass die Krankheitssymptome im allgemein wenig ausgeprägter Natur sind. Es ist nun bemerkenswert, dass hier die ältesten schwartzen Fruchtkörper nicht wie sonst, regelmässig auf die ältesten Blattfiedern verteilt sind, sondern unregelmässig zerstreut vorkommen." Such fronds, showing conidial fructifications, however, rather than "black fruit bodies," have been found also by the present investigator, and one of them, collected at Ringwood, near Ithaca, N. Y., is shown in figure 6. Although its lesions are irregularly scattered, it is noteworthy that the older portions of the frond have many, small lesions, while the younger parts have fewer, larger lesions. This diseased frond bears the closest possible resemblance to fronds artificially inoculated with conidia.

However, in the case of a *lightly* infected field frond showing absence of leaf-roll symptoms and a few leaf spots, it may be impossible to determine from symptoms alone whether the frond has become diseased as a result of inoculation with spores, or whether, as indicated in Part I of this paper, it may have arisen from a systemically diseased rhizome.

Although no inoculated fronds showed any symptoms of systemic infection, yet it seemed theoretically possible that mycelium of the fungus could grow downward through the stipe of a very young frond, reach a rhizome bud, and there initiate systemic infec-

tion. Therefore fronds which appeared subsequent to inoculation in flats used in inoculation experiments were examined, but no frond showing symptoms of systemic infection ever appeared. Nor did any of the fronds show signs of localized infection, except two which came up in the same flat only 3.3 and 4 cm. from inoculated fronds, not long after inoculation. One of these showed a single lesion and the other a number of scattered lesions on the tip of the frond. Their infection was doubtless due to conidia from one of two sources: the soil of the flat which, uncovered at the time of inoculation, had received a rain of conidial suspension, or the conidial masses which oozed from the lesions of the inoculated fronds close by.

2. *Inoculation of Soil and of Underground Parts*

Inoculation of the soil in which healthy bracken plants were growing, and inoculation of underground parts of the bracken exposed by digging or washing, were also tried, using suspensions of freshly collected conidia in sterile distilled water as inoculum. None of these experiments resulted in the systemic infection of the bracken. Inoculation of underground parts exposed by digging or washing resulted in some localized infection of fronds, the lesions being of the large, distorted type characteristic of infection resulting from inoculation of unexpanded frond parts above ground.

3. *Inoculation of Spores and Gametophytes*

Experiments were undertaken to find out whether young bracken plants (sporophytes) become infected with *Cryptomycina Pteridis* through the presence of the fungus upon (or within?) the bracken spores or through inoculation of the gametophytes from which the young sporophytes later arise.

Spores from healthy bracken plants came from Ringwood, near Ithaca, N. Y. Spores from diseased bracken and conidia for inoculum came from diseased fronds in the Smith College Plant House.

When bracken spores were disinfected, the spores were shaken up with filtrate of 10 grams calcium hypochlorite in 125 cc. dis-

tilled water in a 10 mm. test tube, left for 10 to 15 minutes, and transferred by means of a flamed loop from filtrate directly to slant.

The bracken spores were sown on large test tube slants of synthetic medium. Development of gametophytes was followed by development of sporophytes. Whenever it appeared necessary, sterile distilled water was added to the cultures to prevent drying. The experiments were started in October, 1936.

Four slants were sown with undisinfected and three with disinfected bracken spores obtained from *diseased* bracken fronds.

Three slants previously sprayed with a suspension of conidia in sterilized water were sown with undisinfected spores from healthy fronds.

Four slants of young gametophytes (developed from disinfected spores from healthy fronds) were inoculated with a suspension of conidia in sterilized water.

The bracken sporophytes which subsequently developed in these tubes, like the sporophytes which developed in control tubes, showed no symptoms of being infected with *Cryptomycina Pteridis*. There is therefore *no evidence that sporophytes become infected through the gametophyte generation, since no infected sporophytes developed in (1) gametophyte cultures grown from spores of diseased bracken, (2) gametophyte cultures grown from inoculated spores, (3) cultures of inoculated gametophytes.*

4. Inoculation of Young Sporophytes

a. Inoculation of Sporophytes in Test Tubes

Eleven young sporophytes in test tubes (developed in cultures from disinfected spores of healthy bracken) were inoculated with conidia in March, 1936.

By June two plants showed conidial fructifications from which conidial cirrhi were forced out. The conidia were like those of *Cryptomycina Pteridis*, measuring 10–15 $\mu \times$ 4–5 μ. No signs of similar infection appeared on sporophytes of control tubes. The cramped plants were transferred to pots of sterilized humus in July, but did not survive transplanting, and no evidence as to whether or not the infection was systemic was therefore obtained.

Test tube cultures were satisfactory for experiments with

bracken spores and gametophytes. They were unsatisfactory for the growing of bracken sporophytes because of the small number of plants per tube and the difficulty of transferring plants from agar to soil. Moreover, all cultures became contaminated. Contaminants were of course introduced with the inoculum in all the inoculation experiments since the conidia were obtained from the surface of fronds, but control tubes also became contaminated in the course of the months necessary for the experiments, months during which irrigation of the cultures was necessary.

Another method of growing young sporophytes was therefore followed.

b. Inoculation of Sporophytes on Inverted Flower-Pot

Abundant growth of young sporophytes attached to gametophytes was obtained in November, 1937, from bracken spores sown in July on the outer surface of a flower-pot filled with sphagnum, inverted in a shallow dish of water, and covered by a bell glass with open tubulated top.

The sporophytes were small, with the axis still vertical and undivided, the axis with its leaves having a height of about one to two centimeters.

Three areas of pot surface, from edge to bottom, were then stripped of plants, thus leaving three areas of young sporophytes separated from each other by bare areas. The plants in two of the areas were inoculated with a suspension of conidia in sterile distilled water, while those of the third area were left as controls, being sprayed with water only.

Leaf spot lesions developed on the fronds of several plants of the inoculated areas, while none were found on plants of the control area. Conidia from an exuded mass were typical of those of *Cryptomycina Pteridis* in form and size, measuring 11–16 $\mu \times 4 \mu$.

Stained sections of the sporophyte on which the mass of conidia was found showed a fungus parasite in the embryonic region of its stem and in the youngest leaf primordium, as well as in older parts of the plant. The intra- and intercellular mycelium was just like that of *Cryptomycina Pteridis* in buds of mature, systemically infected bracken plants. Only four uninvaded cells separated the

parasite from the apical cell. In several invaded cells the nucleus was undergoing mitosis.

The conclusions drawn from this experiment and those drawn from the next experiment will be summarized together at the end of Part II.

c. Inoculation of Sporophytes in Soil

Three young sporophytes transplanted from pot surface to soil in November were inoculated in April with a suspension of conidia in sterile distilled water, while a fourth plant, the control, was sprayed with water only. In all four plants a portion, at least, of the rhizome system bearing young crosiers was still exposed above the surface of the soil, and the inoculum was directed particularly at the young rhizomes and buds. Some of the inoculum fell on the fronds also, and typical localized frond lesions were later observed on two of the inoculated plants.

In the middle of July, one frond of an inoculated plant showed symptoms of systemic infection, exhibiting the arching of pinnules characteristic of fronds arising from infected rhizomes. A second, younger frond adjacent to the first soon showed the same symptoms, followed by a third. No such symptoms of disease appeared in the two other inoculated plants nor in the control plant.

Typical early symptoms were followed by formation of conidial fructifications, from which conidia oozed. These were typical *Cryptomycina Pteridis* conidia in form, manner of germination, and size, those measured being 9–14.5 $\mu \times$ 3.5–5.5 μ.

Healthy fronds of bracken inoculated with these conidia developed typical localized lesions with typical conidial fructifications and conidia.

Free-hand sections of rachises of pinnae of diseased fronds of the young sporophyte showed the fungus to be present between and within the bracken cells. Its appearance and distribution were the same as the appearance and distribution of *Cryptomycina Pteridis* mycelium in similar sections of a typically diseased frond developed from a diseased rhizome planted in the greenhouse.

When sectioned and stained, the stem bud of the rhizome was found to be infected with mycelium. In the undifferentiated tissue

of the tip only two cells separated the parasite from the apical cell. A sectioned frond bud also showed typical infection.

The results of this experiment and of the preceding show that *(1) a systemic infection of bracken, characterized by the presence of a parasite in the undifferentiated tissues of stem buds and leaf primordia, and by the development of diseased fronds from diseased leaf buds, may be initiated in young sporophytes by inoculation with conidia of Cryptomycina Pteridis, and that (2) this disease is identical with the leaf-roll disease of bracken, and that the parasitic fungus which causes it is identical with Cryptomycina Pteridis, the cause of leaf-roll disease.*

PART III. STUDIES ON PENETRATION AND EARLY STAGES OF INFECTION

It will be recalled that the attempts of Killian (**1918**) to germinate ascospores on pinnules of bracken in moist chambers met with failure, as did also attempts to germinate conidia, and that his conclusions as to the way in which *C. Pteridis* gains entrance into its host were based on study of fronds ". . . die in die Natur unter ganz normalen Bedingungen der Infection durch den Parasiten ausgesetzt gewesen waren." He found attached, germinating spores on both surfaces of the frond, but no evidence of penetration, and he interpreted as ascospores germinating in sub-stomatal chambers structures which his accurate drawings indicate to be simply thickened branches of mycelium in diseased fronds which were undoubtedly developed from diseased buds of systemically infected bracken plants. Similarly, his excellent illustrations and in most points perfectly accurate descriptions of "early" stages of frond infection do not apply to early stages at all, but to intermediate stages in the development of a systemic mycelium. (This point has been more fully discussed in Part I.)

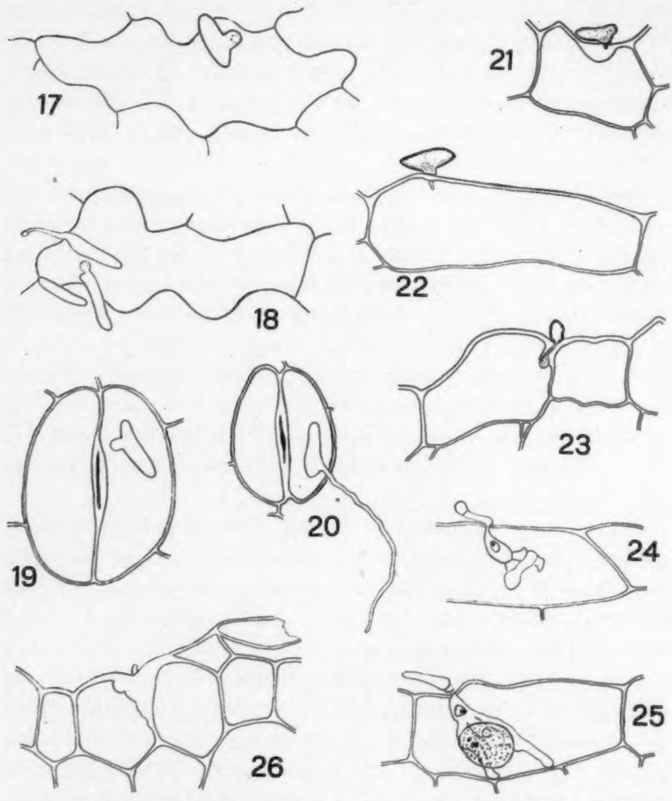
With the systemic nature of the disease demonstrated, it becomes clear that reliable data on penetration and early stages of infection can be gathered only from studies of artificially inoculated fronds from healthy rhizomes. Therefore, young fronds of healthy bracken in the greenhouse were sprayed with conidia of *C. Pteridis* in sterile distilled water. Small pieces of inoculated pinnules for sectioning were fixed in formalin-acetic-alcohol after 8 hours, 24

hours, 2, 3, and 4 days; while large pieces for surface examination were fixed after 2 days in a mixture of equal parts of acetic acid and 95 per cent alcohol. The large pieces were cleared in chloral hydrate, washed, and stained with cotton blue (12 minutes) or lactic blue (30 minutes). The better staining was obtained with cotton blue.

Examination of frond surfaces showed many germinating conidia on both surfaces of the frond. Their germ tubes varied in length. They were as likely to grow away from stomata as toward them (FIGS. 19, 20), and one tube was seen growing across a stoma. The germ tubes often followed the lines of radial walls of the epidermal cells. Frequently the germ tubes grew toward germ tubes of other conidia of the fungus. A small, brownish ring could usually be distinguished, outlining an area of the tube which was closely appressed to the surface of the host (FIGS. 17, 18). This area of the germ tube will be referred to as the appressorium.

Stained microtome sections showed that after 8 hours many conidia had germinated and formed appressoria. After 24 hours, penetration of the epidermal wall was taking place or had just been accomplished, by means of a minute peg-like outgrowth from the appressorium. Such a penetration tube, growing into a radial wall, is seen in figure 21. (The section shows only a portion of the obliquely cut wall.) In figure 22, the penetration tube has reached the lumen of the cell through the outer epidermal wall, which has become slightly thickened in the region pierced. Figure 23 shows a longer penetration tube which has grown within the radial walls of two adjacent epidermal cells and then sideways into one of these cells, where a vesicular swelling has formed at the tip of the penetration tube. Cells of both the upper and lower epidermis were penetrated by the fungus.

Sections of material fixed 2 days after inoculation in some cases showed large vesicular swellings of the mycelium of the parasite within the penetrated cell. In other cases, considerable branching of the mycelium within the host cell had taken place, as illustrated by figure 25, where several haustorial branches are in contact with the nucleus of the host cell. In still other cases the fungus had failed to complete penetration of the wall of the epidermal cell of



FIGS. 17, 18, germinating conidia of *Cryptomycina Pteridis* on upper epidermis of young bracken frond, surface view; 19, 20, germinating conidia on guard cells of lower epidermis, surface view; 21, conidium with penetration tube in radial wall of epidermis, cross section, 24 hours after inoculation; 22, conidium with penetration tube which has pierced outer wall of epidermal cell, 24 hours after inoculation; 23, conidium with penetration tube and vesicular swelling, 24 hours after inoculation; 24, vesicular swelling with hyphal branches, 2 days after inoculation; 25, hyphae in contact with nucleus of host cell, 2 days after inoculation; 26, incomplete penetration, radial wall of epidermis thickened and discolored, 4 days after inoculation. All figures about × 700.

the host, and this failure was apparently correlated with abnormal changes in the cell wall, which had become thick and dark in color below the appressorium (FIG. 26). It also appears probable that when the fungus has grown through the epidermal wall it is sometimes unable to spread beyond the first cell invaded. This is indicated by certain sections of material fixed 4 days after inoculation which show a thick, dark sheath around the parasite, which is still confined to a single host cell.

When invasion is successful, however, the fungus grows rapidly from cell to cell, being found in material fixed 4 days after inoculation as far as the fifth row of cells from the surface of the invaded epidermis. Since the leaf was 8 cells in thickness in this region, the mycelium had advanced more than half way through the leaf.

In the preparation of material for the above study of germination and early stages of infection, only conidia were used as inoculum. For reasons already stated, ascospores are obtainable only in late spring and early summer and it is difficult to obtain ascospore inoculum uncontaminated with conidia. However, experiments with ascospores are in progress and will be reported on in a later paper.

SUMMARY

Cryptomycina Pteridis (Rebentisch ex Fries) von Höhnel, cause of leaf-roll of bracken, was studied in its relationship to *Pteridium latiusculum* (Desv.) Hieron ex R. E. Fries, the eastern bracken.

1. The fungus is systemic and perennial, overwintering in stem buds and frond buds of its host, and persisting indefinitely in a given diseased plant.

2. The mycelium of the parasite was not found in the apical cell of the stem bud, but in the undifferentiated tissue adjacent to it, separated from it by from 2 to 11 uninvaded cells. The mycelium is both inter- and intracellular. It was occasionally seen in a bracken cell the nucleus of which was undergoing mitosis.

3. In the maturing rhizome, which gives no external evidence of being diseased, the scattered infection areas in the various tissues remain of limited extent, and the fungus apparently dies. In the maturing frond, which exhibits striking symptoms of being

diseased, the fungus spreads from originally scattered foci and develops with marked luxuriance.

4. Typical symptoms and signs of leaf-roll disease are found only in fronds developed from diseased buds of systemically infected plants.

5. Inoculation, with conidia, of young fronds or of immature portions of older fronds was followed by infection resulting in localized lesions.

6. Inoculation, with conidia, of young bracken sporophytes was followed by infection resulting in systemic infection and typical leaf-roll symptoms.

7. No systemic infection of mature plants followed the inoculation, with conidia, of (1) their fronds, or (2) the soil covering their underground parts, or (3) the underground parts themselves.

8. No systemic infection of young sporophytes followed the inoculation, with conidia, of bracken spores or young gametophytes.

9. Germination of conidia was obtained in various liquids and on the surface of young bracken fronds.

10. The fungus enters the host by sending a penetration tube through the wall of an epidermal cell. The hyphae grow rapidly from cell to cell.

Specimens of material used in the above experiments and observations have been deposited in the Herbarium of the Department of Plant Pathology of Cornell University, the Farlow Herbarium of Harvard University and the Herbarium of the Bureau of Plant Industry.

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MYCOLOGICAL NOTES FOR 1936–38 [1]

L. O. Overholts

(WITH 14 FIGURES)

This paper is a continuation of a series with similar title begun in 1919 and continued until the present time. They represent an attempt to throw light on the new and unusual fungi encountered from year to year, especially those inadequately known or lacking complete descriptions and illustrations.

ASCOMYCETES

APOSTEMIDIUM VIBRISSEOIDES (PECK) BOUD. (FIG. 3)

Although I had searched for this fungus for years in central Pennsylvania, it was not collected until the summer of 1938, and then only in limited amounts. Since Durand lists it only from New Hampshire, Vermont, and New York, it is likely that Pennsylvania is on the southern limits of its range. I have seen no published photo of an American collection.

CENANGIUM GRISEUM DEARN. & HOUSE (FIG. 8)

Collected at Laurel Run, Huntingdon County, Pa., June 30, 1938, on dead *Acer rubrum*. The original host was *A. spicatum*. I collected this fungus in Ontario with Dr. H. S. Jackson. On securing the Pennsylvania material I suspected the identity of the fungus and sent part of it to Dr. Dearness, who verified the determination. The species was originally described from Ontario. In my Pennsylvania material the disk of the apothecium is much more olivaceous tinged than in the Ontario material.

[1] Authorized for publication on May 16, 1939, as paper no. 906 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Contribution no. 126, Department of Botany, The Pennsylvania State College, State College, Pa.

CORYNETES ROBUSTA DURAND (FIG. 1)

When Durand wrote his monograph on Geoglossaceae in 1908 he reported this species from only Maine, Massachusetts, New York, North Carolina and Mississippi. In the summer of 1937 it was abundant in one locality along Stone Creek, Huntingdon County, Pa. Appearing about August 18, the specimens developed slowly until about September 10. Although the spot is one visited

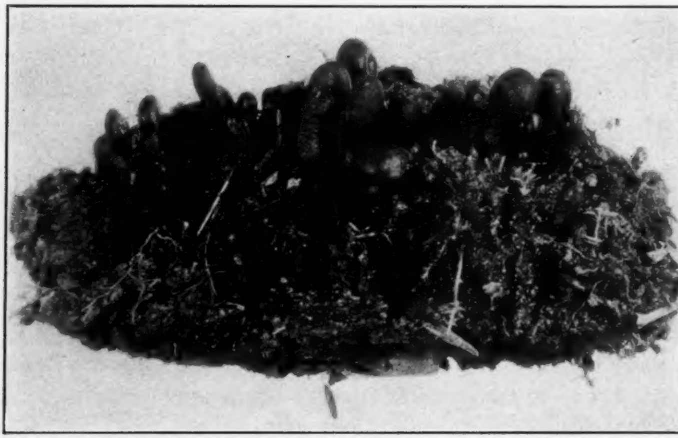

FIG. 1. *Corynetes robusta.* × 1.

several times in late summer and fall collecting every year, the fungus had never previously been seen there and it did not reappear in the season of 1938. Further evidence of the rarity of the species of *Corynetes* is the apparent fact that Peck never collected or at least never reported anything under that generic name. The photo presented herewith is of plants at the time the colony was first discovered—hence quite immature. I have a photo that would duplicate Durand's illustration.

MYCOSPHAERELLA SARRACENIAE (SCHW.) HOUSE

Perithecia crowded over the stem surface, appearing as small black emergences without definite arrangement, 50–70 μ diameter; asci pyriform to ellipsoid or subglobose, 18–24 × 12–14 μ, 8-

spored; spores oblong or somewhat narrowed at one end, smooth, hyaline, 2-celled, 12–16 × 3.5–4 μ.

On dead flowering stalks of *Sarracenia purpurea*. Lopez, Sullivan County, Pa., September 11, 1936. The species was originally described from the Carolinas. House (1921) reported it from New York, on both leaves and peduncles. I know of no other reports of its occurrence. I find the spores to be somewhat larger than in the original description.

TAPHRINA FILICINA ROSTR.

Recent advances in our knowledge of the species of this genus have been considerable. The above species is listed by Mix as known only from the vicinity of Ithaca, New York. A collection made in McKean County, Pa., on *Aspidium spinulosum* in 1938 was sent to Mix and he pronounced it as correctly determined. The fungus was abundant over a considerable area in the McKean Forest, near Mt. Jewett, Pa.

FUNGI IMPERFECTI

Cercospora Polemonii sp nov.

Spots indefinite, bounded by the larger veins, occupying a large part of the leaf surface; conidia hypophyllous, appearing under a lens as a fine smoky scurf, the conidiophores fasciculate, short, 20–30 × 5–5 μ, brownish; spores elongate, subhyaline to somewhat smoky, 1-celled then up to 4-celled, straight, clavate-cylindric, 32–60 × 2.5–5 μ.

The type of this species was collected on living leaves of *Polemonium reptans*, State College, Pa., September 19, 1912, by J. B. Demaree. Overholts Herbarium *11690*.

This is the collection reported in Mycologia **30**: 269. 1938 as *C. omphakodes*. Dr. Charles Chupp informs me that reference could hardly be correct.

CORNULARIA PERSICAE (SCHW.) SACC.

Pycnidia erect, spiniform, flexible when wet, rigid and brittle when dry, black, 800–1000 μ high, about 125 μ diameter, under the microscope the walls roughened by the protruding ends of narrow, brown or blackish hyphal tips; conidia elongated, straight or slightly

curved, 7–10-celled, brownish except for the hyaline terminal cells, 64–80 × 3.5–4 μ.

On dead branches of *Prunus* sp. (probably a plum). State College. Pa., December 15, 1937. A most inconspicuous fungus that I am not able to enlarge enough to present a photo. Apparently rare.

EXOSPORIUM TILIAE LINK (FIG. 7)

Stromata 0.5–1 mm. diameter and about as high, erumpent, subglobose to depressed-globose and somewhat pezizaform, black, appearing powdery, waxy when wet, becoming hard on drying; in section composed of nearly colorless hyphae, with a definite black cuticular layer made up mainly of the blunt conidophores 10–12 μ diameter and about as high, dark-colored; conidia clavate, dark colored, 60–80 × 16–17 μ, indistinctly transversely septated into a number of cells, the walls very thick.

On dead branches of *Tilia.* Collected at the foot of Mt. Davis, Somerset County, Pa., July 15, 1938. Though listed in the Seymour Index, I have seen no other reference to this fungus in America. Superficially and without magnification it resembles *Strumella,* but under a lens the stromata are more firm and decidedly cupuliform in many cases, simulating a *Cenangium.*

PHYLLOSTICTA GUTTULATA HALSTED

Seaver reports this species from only Vermont and New York. A collection was made at State College, Pa., September 19, 1931, on *Oxalis stricta.* The pycnidia are unusually large, measuring up to 200 μ diameter.

PHYLLOSTICTA MACROSPORA ELLIS & EV.

On living leaves of *Liriodendron Tulipifera* there is a common spot, perhaps of insect origin, that serves as a substratum for at least four different species of fungi in Pennsylvania. A species of *Cladosporium* forms a white floccose pubescence on the lower surfaces of some of the spots. A *Phyllosticta* which seems referable to *P. liriodendrica* is sometimes present with elliptic spores 5–8 × 3–5 μ. Again there is sometimes present a spermogonial stage of *Mycosphaerella Liriodendri* (Cooke) which seems never

to have been transferred from the old genus *Sphaerella*, and of which *Phyllosticta liriodendrica* has been said to be the conidial stage. There is also at times another *Phyllosticta* with spores 15–17 (–22) × 4–6 μ. This agrees with the spore measurements of *P. macrospora*. That species, however, Seaver (N. Am. Flora **6**: 44. 1922) thinks, may have been based on an immature ascus stage of the *Mycosphaerella*. However, I have demonstrated

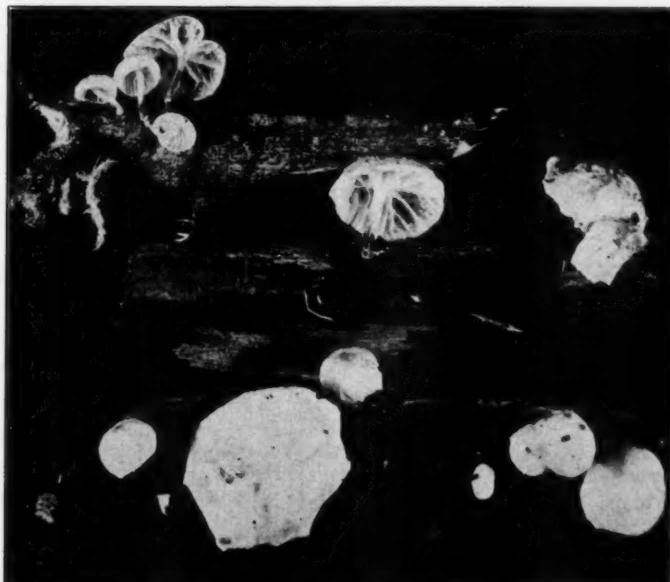

FIG. 2. *Marasmius magnisporus*. × 1.

clearly that there is on these spots a large spored *Phyllosticta* which would answer the description of *M. macrospora*. It is quite possible that the type collection of that species may have included two fungi and that in examining the collection Seaver may have obtained only the immature ascus stage of the *Mycosphaerella*. Collections were made in Center County, Pa., in 1938.

RAMULARIA OXALIDIS FARLOW

Spots circular, elliptic, 3–5 × 2–4 mm., single on the leaflets, the center grayish, with a rather broad, red-brown, conspicuous margin; conidiophores hypophyllous as a thin white pubescence, densely fasciculate, 50–70 × 2–3 μ, septate, hyaline; conidia elongate, 1-celled then mostly 2-celled, occasionally 3-celled, 14–24 × 3–4 μ.

On living leaves of *Oxalis acetosella.* Although a spot had been observed on leaves of this host for many years, no fruiting was collected until 1938 when it was found in quantity near Halsey, McKean County, Pa. I have seen some evidence that this fungus would be better referred to *Septocylindrium,* since in a few cases the spores were seen in chains of two. This point needs further investigation. The original description referred to the rarity of septated spores but I find the septa well developed and conspicuous. The species was originally reported from New Hampshire on this host. The only other record I have seen is that of Dr. Davis from Wisconsin on an unspecified species of *Oxalis.*

Septoria longispora sp. nov.

Spots narrow-elliptic or somewhat elongate, 5–7 mm. long, 1–2 mm. broad, not coalescing, somewhat more conspicuous from above, uniformly brown, scarcely becoming pallid, definite, and with a slightly darker margin; pycnidia minute, 60–80 μ diameter, black, poorly dfferentiated, epiphyllous, scattered; spores linear, smooth, hyaline, 40–95 × 1.5 μ, apparently 4-celled but the septa indistinct.

On living leaves of *Aquilegia canadensis.* Type collected at State College, Pa., September 15, 1931. W. L. White. Overholts Herbarium *21450.*

The spores are much too long for any species listed as occurring on this or related hosts.

SEPTORIA SPICULOSA ELLIS & HOLW.

The original description can be supplemented as follows: spots irregularly elongate, equally visible from both surfaces, 1–2 cm. long, 5–8 mm. broad, smoky brown, not sharply delmited; pycnidia epiphyllus; spores 25–35 × 1.5 μ, and about the size and shape of the cell crystals of the host.

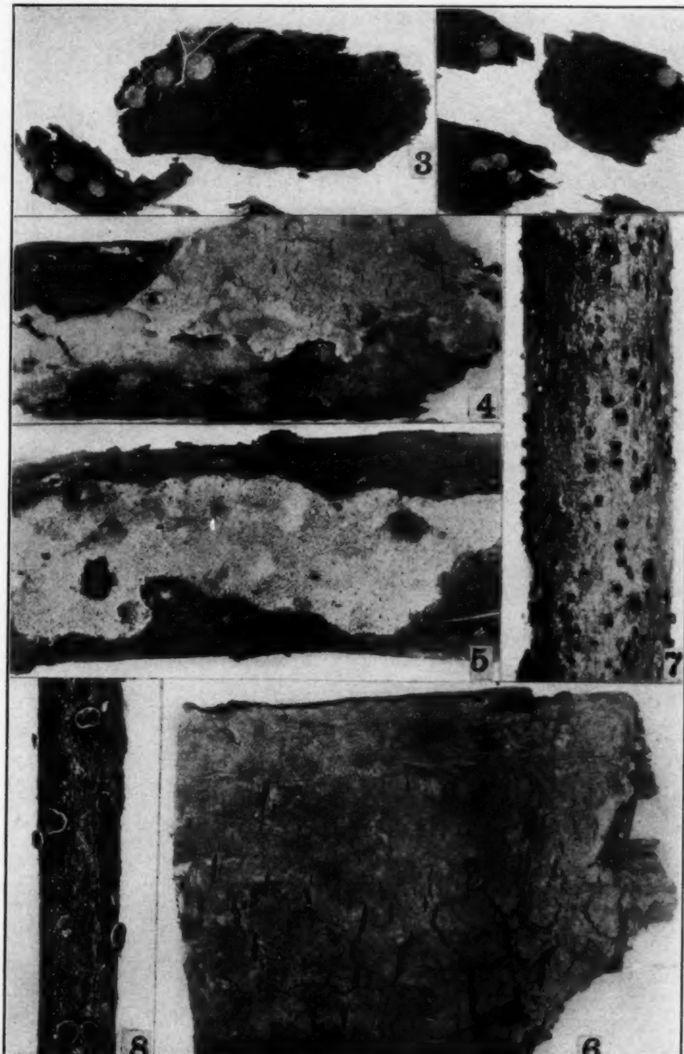

FIG. 3. *Apostemidium vibrisseoides*, × 1½; 4–6, *Poria albobrunnea*, × 1; 7, *Exosporium Tiliae*, × 1½; 8, *Cenangium griseum*, × 1½.

On yellow leaves of *Symplocarpus foetidus.* Charter Oak, Huntingdon County, Pa., July 13, 1930. The fungus is accompanied by a species of *Vermicularia.* Originally described from Wisconsin and Dr. Davis included it in his reports from that state. I have seen no other references to its occurrence, hence this collection extends its range considerably.

SPHAERONEMA MAGNOLIAE PECK

The original description can be supplemented as follows: pycnidia 1–2 mm. long; conidia hyaline, $9–10 \times 5–6\ \mu$; conidiophores hyaline, slender, $20–40 \times 2–3\ \mu$, tapering to the apex.

On dead twigs of *Magniolia acuminata,* Laurel Run, Huntingdon County, Pa., March 13, 1936. The species was originally described from New York. I have seen no other references to its distribution.

THYRSIDIUM HEDERICOLA VAR. CARPINI SACC.

Fruiting structures in the form of mound-like, soft-gelatinous masses 1–2 mm. diameter, or confluent and larger; in section composed of hyaline, erect or suberect hyphae, simple, each bearing a rounded blackish head of spores, $30–40\ \mu$ diameter; spores held together in a gelatinous ball, globose, smooth, olivaceous, $3.5–4.5\ \mu$ diameter, borne in chains.

On dead branches of *Carpinus caroliniana.* Mt. Davis, Somerset County, Pa., July 15, 1938. The Seymour Index lists this species but otherwise I have seen no American records of it. It is a curious fungus. The catenulate arrangement of the spores within the heads is not apparent yet must be inferred when the very young heads are compared with mature ones.

BASIDIOMYCETES

BOLETUS MIRABILIS MURRILL (FIGS. 13, 14)

Collected at Ross Run, Huntingdon County, Pa., September 6, 1937, on the ground under white pine trees. This species is known otherwise only from Manitoba and the Pacific Coast, writes Dr. Snell to whom specimens were sent for identification. The spores measure $20–26 \times 8–9\ \mu$. The very dark red pileus that is strongly viscid (Van Dyke Red or Madder Brown, R.), the rugose or papil-

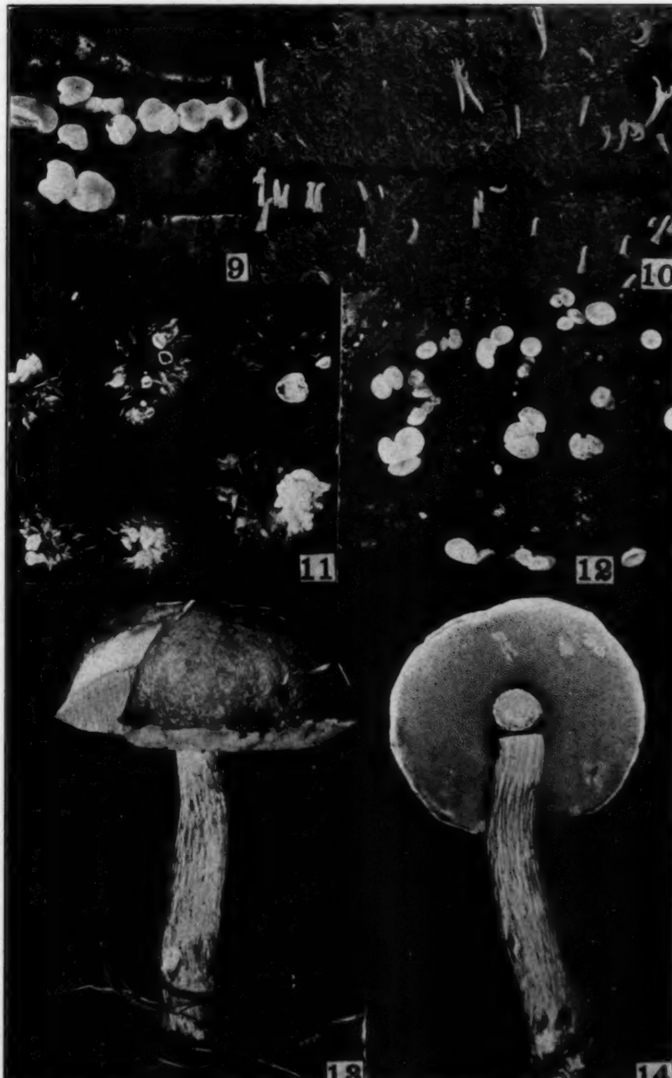

FIG. 9. *Femsjonia luteoalba*, × 1; 10, *Craterellus cristatus*, × ¾; 11, *Cyphella muscigena*, × 1½; 12, *Guepinia pennsylvanica*, × 1½; 13, 14, *Boletus mirabilis*, × ¾.

late pileus, the long narrow reticulations and the pinkish color of the stem are the diagnostic features. The stem reticulations are well developed and this with the pinkish color is strongly reminiscent of such species as *B. Betulae* of the Laceripedes group.

CRATERELLUS CRISTATUS KAUFF. (FIG. 10)

Sporophore clavate or subcylindric, erect, 7–15 mm. high, simple or occasionally branched once or twice near the apex which is expanded and truncate or with 2 to 4 coronate tips, color purplish gray, soft and fleshy, glabrous, narrowed downward, gregarious; spores short-cylindric, smooth, hyaline, 5–6 × 2.5–3 μ; gloeocystidia numerous, appearing as the projecting tips of long flexuous conducting cells 4–8 μ diameter.

On mossy coniferous log. Camp Mercier, Laurentides National Park, Quebec, August 27, 1938. R. F. Cain.

Whether this properly belongs in *Clavaria* or in *Craterellus* is a question. The situation is akin to that of the proper disposition of *Clavaria pistillaris* which has been placed in both genera. My specimens are smaller than those described by Kauffman who found the sporophores up to 2.5 cm. high. Otherwise the agreement is excellent. Kauffman's specimens were collected in Oregon.

CYPHELLA MUSCIGENA FRIES (FIG. 11)

Sporophore grayish-white to isabelline, campanulate to inversely conic, 1–5 mm. diameter, the pileus sessile and attached by the vertex, finally becoming expanded; hymenium concolorous, smooth, or in large plants slightly rugose, the entire sporophore thin, pliant; spores oblong-ellipsoid, smooth, hyaline, 7–10 × 3.5–5 μ; cystidia none; basidia 18–20 × 6–7 μ, 4-spored.

On living mosses, *e.g., Bryum roseum*. A collection of this unique fungus was made at Slippery Rock, Butler County, Pa., in 1938. The sporophore seems to be attached underneath the crown of leaves of the *Bryum* plants, where it is pendent, apparently attached at or near the leaf bases. In the Pennsylvania plants there is no indication of a stem. Bourdot and Galzin say that Burt's plants under this name cannot represent the species, differing in structure and in smaller spores. Burt gives the spores as 4.5–5 × 2.5–3 μ and says these measurements follow those of European

exsiccati and likewise of Bresadola. But Bourdot and Galzin say that Bresadola's description in Fungi Polonoci apply in part to *Arrhenia auriscalpium*. They give the spore measurements as 7.5–9–12 × 4.5μ6 μ, with which my plants agree well.

Guepinia pennsylvanica sp. nov. (FIG. 12)

Sporophores single or more frequently in clusters of 2 to 6, cupulate and rather distinctly stipitate, 2–5 mm. broad, yellow or ochraceous-orange, darker when dried and paling when repeatedly soaked up, 5–6 mm. high; hymenium smooth; stem externally ribbed; exterior of sporophore provided with an irregular palisade of inflated, thick-walled cells with very narrow lumen, these apparently originating as systems of apical branchings or perhaps at times as the tips of single hyphae, often septated, and provided over their exteriors with a rough coating of spines or a definite shagginess, the walls and spinules unstained in phloxine, 8–12 μ diameter; spores elongate, often somewhat curved, hyaline, one-celled, then finally 4-celled, but one-celled spores usually predominating, smooth, 9–14 × 4–5 μ.

On bark of dead standing Betula. Type collected at Ross Run, Huntingdon County, Pa., June 15, 1936. In general appearance the species is much like *G. Peziza* (= *G. tortus*) but differs in the shorter and much more roughened cortical (peridial) cells and the broader spores. I see no special merit in recognizing the segregation of *Guepiniopsis* from *Guepinia*.

FEMSJONIA LUTEOALBA FRIES (FIG. 9)

Until recently this fungus was unknown to America. Brasfield recently reported it from New Hampshire, Ontario, and Ohio. I would suspect that the Ohio report is an error, not in identification, but in the origin of the collection. There is too much difference between the Ohio climate and the climate under which I have seen this fungus so abundantly in Ontario and in Quebec. This opinion is supported by the apparent absence of the species from the Appalachian region, where the environmental factors would approach much more nearly those of its more northern range. Specimens I have seen in the field are more nearly a pale buff than an orange yellow as described by Brasfield, although the orange color of the

hymenium may extend over the remainder of the sporophore to some extent. The only substratum from which I have collected it is *Betula*. It was abundant in the region of Duchesnay, Quebec, in August, 1938. This is an extension of the previously known range. I have additional collections from Ontario.

MARASMIUS MAGNISPORUS MURRILL (FIG. 2)

Collected at Laurel Run, Huntingdon County, Pa., August 11, 1937, on dead outer bark of living *Ulmus americana*. The species is none too well described, nor does it key out well in Pennington's treatment of the genus. I present, therefore, the description drawn from the 1937 collection:

Pileus 5–15 (–25) mm. broad, reviving completely after drying, convex to convex-campanulate, white, appearing tomentulose or appressed-cottony under a lens, rather delicate and appearing subgelatinous when water-soaked, drying white; context very thin, taste mild; gills adnate or somewhat decurrent, strongly interveined, forked, white, about 1 mm. broad; stem central or somewhat excentric (one specimen practically lateral), curved, at first white and minutely pubescent under a lens, at maturity somewhat blackish toward the base, 5–7 mm. long, 0.5–1 mm. thick; spores tear-shaped, *i.e.*, pip-shaped with an unusually long apiculus, smooth, hyaline, 10–16 $\times$ 4–5 μ; cystidia none.

Pennington uses the upward flaring of the stem as a key character. In my specimens this is not pronounced and certainly should not be used as a key character; the gills are not decidedly decurrent in all specimens; the spores run considerably larger than in the descriptions. Apparently the species is not so rare. Hard's figure 107, as *M. candidus*, is apparently this species.

PORIA ALBOBRUNNEA ROMELL (FIGS. 4, 5, 6)

Broadly effused, sometimes for 10–20 cm. on decorticated wood, the general color pallid brown in herbarium specimens; annual, 1–3 mm. thick, separable, first developing as orbicular patches of a distinct felt-like subiculum which is gray at first but often becomes brown, and in mature specimens is visible only as a distinct marginal zone of compact tomentum 1–5 mm. or more broad, or at times practically disappearing; subiculum persisting as a soft whitish cottony layer scarcely visible to the unaided eye; tubes 0.5–3

mm. long, whitish or pale wood-colored within, the mouths varying from pale drab gray to avellaneous or wood brown in herbarium specimens, at first subcircular, finally more or less hexagonal, the dissepiments rather thin, sometimes slightly pubescent under a lens, very even and entire, averaging 3.5 to 6 per mm., the hymenium perhaps typically cracked with age; spores cylindric or allantoid, hyaline $4–6 \times 1.5–2\ \mu$; cystidia none; hyphae of the margin hyaline or hyaline and brown mixed, simple or sparingly branched, with very few cross walls and clamps and often these apparently absent, $2.5–4.5\ \mu$ diameter; in KOH solution sections of the hymenial region develop a sulphur-yellow color in the basidial layer and sage-green in the trama.

On decorticated wood of *Pinus monticola*. Known only from the vicinity of Priest River, Idaho. In my herbarium there are portions of four collections communicated to me by Dr. Weir many years ago. Parts of these specimens were submitted to Pilat at Prague, and he verified the determination. I also have had a specimen from Romell for comparison. Since there is no change to blackish when KOH is applied this species is not to be referred to the group of the brown Porias. It has not previously been reported from America. The fungus is associated with a brown carbonizing decay of the wood.

PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PA.

NOTES AND BRIEF ARTICLES

CORRECTION

In the list of Myxomycetes from Quebec published on pages 728–729 in the November–December 1939 issue the species *Physarum aureum* Brandza and *Physarum sessile* Brandza should be omitted, and *Physarum sulphureum* Alb. & Schw. var. *sessile* should be added. The matter is more fully discussed on pages 346–348 in the May–June 1939 issue.—Robert Hagelstein.

Heterothallism in Ascobolus geophilus

Ascobolus geophilus was first collected by the writer while a student in the University of Iowa. It was found rather abundantly on mud flats along the Iowa River, and was referred to as *Ascobolus viridis.* Later studies convinced the writer that it was distinct, and it was described as a new species based on material collected in The New York Botanical Garden. It has been frequently collected since in various parts of the country. Edwin M. Betts and Samuel L. Meyer have recently taken up a study of the species and found it to be heterothallic in that they cannot produce apothecia from the germination of a single spore, but only after it has been crossed with another spore of the opposite strain. The results of this work have been published in the American Journal of Botany **26**: 617–619. 1939.—F. J. Seaver.

Fungi of the Duke Forest

Duke University is unusually fortunate in having a large tract of woodland, comprising more than 3000 acres, just outside their door and, in fact, almost surrounding the University campus, which constitutes an outdoor laboratory for the study of practical forestry and allied subjects. Dr. Frederick A. Wolf and collaborators have taken advantage of this opportunity to conduct studies in forest

pathology and forest mycology. Bulletin 2 of Duke University School of Forestry consists of a record of all the fungi collected in Duke Forest over a period of six years. The Bulletin is thoroughly illustrated and consists of 122 pages, comprising both an index to the fungi collected and a host index, and will be found extremely useful to both mycologists and foresters.—FRED J. SEAVER.

In a collecting trip through Florida and the adjoining states during the winter of 1939, I collected a rust on *Oxalis Martiana* Zucc., at Brunswick, Georgia. It was identified as *Puccinia Oxalidis* (Lev.) Dietel & Ellis. In the Manual of the Rusts in United States and Canada the range for this rust is given as southern Florida, southern Louisiana, New Mexico, Texas and Mexico. This collection extends the range farther north. The identification of the rust was checked by Dr. F. D. Kern of Pennsylvania State College.—DAVID R. SUMSTINE.

DISTRIBUTION OF A SLIME-MOLD

A noteworthy example of plant distribution occurs in the case of *Diachea miyazakiensis* Emoto. It is probably superfluous to say that this organism is one of the slime-molds technically called Myxomycetes, a word meaning mucous-mushrooms, and by other authorities Mycetozoa, literally mushroom-animals because in pursuit of food they act like animals, while in their reproduction by spores they might be taken for minute puff-balls, many of them very attractive in form and color.

Japan must have been diligently searched for Myxos seeing that by Emoto's count it was in 1934 the home of 234 species, while by the writer's counting Ontario with two and a half times the area of Japan had a record of 154 species, the United States 281 species and the world at large 373 species. In spite of careful searching the *Diachea* mentioned was not recorded until 1935 anywhere else in the world than in a locality near Tokio.

Last year Mr. Eli Davis picked up a Myxo near London, Ont., and this year another near Acton, Ont., both of which on recent

examination prove to have the same singular capillitial structure and other features of Y. Emoto's *Diachea*. The two Ontario localities are 91 miles apart, and both over 9,000 miles west or over 13,000 miles east from the Japan locality.

It will pass without argument that the species must have originated in a locality somewhere; and in generation after generation has either crossed or skirted around the Atlantic or the Pacific Ocean. The reader is invited to exercise his imagination upon the time that has elapsed since the trek began, or the miles traversed, and marvel that the microscopist cannot find, after an undoubtedly long separation in time and space, any discrepancy between Emoto's presumably good description and the fungus features of the Ontario Myxo which he sees under the microscope.

In his interesting book on Bats, G. M. Allen states that fossil remains of perfectly good bats date back to Eocene times and that as a distinct order of mammals they have continued through the intervening sixty million years to the present day. Myxos could thrive in an environment much more primitive than bats. It is hardly conceivable that a complete fossil of a slime-mold of Eocene date can be in existence, but it is quite conceivable that slime-molds in considerable variety of form and color existed before bats developed the wonderful mechanism enabling them to take their flying food in the air. We can therefore reasonably allow *Diachea miyazakiensis* 60,000,000 years or possibly twice that period to move from the place of origin to reach Japan and Canada.—JOHN DEARNESS.

TAPHRINA CARVERI RECENTLY DISCOVERED IN MISSOURI

(WITH 1 FIGURE)

A specimen of *Taphrina* on silver or white maple (*Acer saccharinum* L.) collected at Lutesville, Bollinger Co., on May 23, 1939, by Mr. Linder Englehart, was recently received from Prof. W. E. Maneval, of the University of Missouri. A critical examination of the specimen shows that the species concerned is *Taphrina Carveri* Jenkins [1] recently described on this kind of maple.

[1] Jenkins, A. E. New species of *Taphrina* on red maple and on silver maple. Jour. Wash. Acad. Sci. **29**: 222–230. 1939.

The specimen just cited is of particular interest since it represents not only an additional new State for the distribution of this fungus, but also it is the first observation of this species in the field since 1897. Moreover, as cited elsewhere, only three previous gather-

FIG. 1. *Taphrina Carveri* on *Acer saccharinum*. × 500.

ings of the fungus are known, viz., Ontario, 1893, and Alabama and Michigan, 1897. In connection with the distribution of the fungus in Missouri, it may be recalled that the young trees on which Dr. G. W. Carver discovered the disease in Alabama were said to be from a nursery in the neighboring State of Iowa.[2] The photograph of *T. Carveri* here shown is from the type specimen, which is the gathering from Michigan.—ANNA E. JENKINS.

OTHER POISONINGS WITH CLITOCYBE ILLUDENS

An account of one case of poisoning from the above named fungus was recorded in Mycologia **31**: 110. Recently a second case has come to our attention involving the poisoning of three individuals.

On September 27, 1939, a fungus referred to the writer by the Microanalyst of the Department of Health in New York City was identified as our old offender, *Clitocybe illudens*. The following detailed report on the case was later received:

> "The mushrooms were picked on Sunday morning, Sept. 17, 1939, by Mr. F. an Italian resident of the Bronx, at Kensico Dam. He shared half of them, about 3 pounds, with Mr. A., his tenant. Mrs. A. prepared them for supper at about 6 P.M. Mr. and Mrs. A. consumed but a few spoonfuls because the taste was not as it should be.

[2] Loc. cit. See footnote 1.

"On Monday, Sept. 18, 1939, Mrs. A. took sick at 8:30 A.M., and Mr. A. at about 9:00 A.M. They vomited, had diarrhea, and were in a weak condition. A physician was called in and they were both taken to a hospital. Mr. A. was sent home as his case was not a severe one. However Mrs. A. was kept hospitalized until Sept. 20, 1939, and then released.

"Mr. F. who had intended to eat his mushrooms for lunch on Monday, Sept. 18, 1939, was informed of the sickness of Mr. and Mrs. A. But he doubted that the mushrooms were the cause, and as an experiment he tried three and consumed them. He vomited within five minutes and then took a large glass of epsom salts. He required no medical attention."

Among the mildly poisonous mushrooms which would include those which do not cause death, although they may bring about severe illness of longer or shorter duration, *Clitocybe illudens* seems to be one of the chief offenders. The reason doubtless lies in the fact that the fungus occurs in such profusion, is so beautifully colored, and looks so good, it is not surprising that unsophisticated collectors should want to feast upon it, and as McIlvaine has aptly expressed it "turn from it with a regret that lingers." May we add that it is much better to have this regret before eating than after, since the after regrets are likely to linger even longer, as indicated by the above experiences.

These records are published as a warning to over-enthusiastic mushroom collectors, not to allow their appetites to triumph over their better judgment in selecting forms to be used as food. "When in doubt throw it out" is a good slogan to be followed by either the amateur or professional mycophagist.—F. J. SEAVER.

TYROMYCES GRAMINICOLA

Stewardson Brown and N. L. Britton collected a polypore in a clump of grass near Harrington Sound, Bermuda, in 1912, which Dr. Murrill (Tropical Polypores 21. 1915.) described as a new species under the name *Tyromyces graminicola*. Dr. Murrill suggested that the host might be a species of *Sporobolus*. So far as known this is the only collection of this species until twenty-six years later in 1938, when Seaver and Waterston found it again

near the Lighthouse on stump of pampas grass, *Cortaderia argentea* (Nees) Stapf. A third collection was made by Mr. Waterston in November, 1939, on a clump of grass under trees in the grounds of the Public Buildings, Hamilton. So far the species is not known outside of Bermuda, but it is probable that it might be found in Florida.—DAVID R. SUMSTINE.

FURTHER NOTES ON DOUBLE COVER-GLASS MOUNTS

The double cover-glass mount described by Diehl [1] should be more generally used for mounting fungi, algae, and other non-embedded microscopic material than it now seems to be. Mounts are made quickly, easily, and are relatively permanent.

The reason for its present limited use may be due to the fact that Diehl confined his explanation wholly to the manner of sealing and without further directions it is difficult to procure a mounting fluid that is compatible with the xylol in the balsam. A beginner, instead of procuring a perfectly clear mount, is likely to be disgusted with the milky-white opaque slide that results when water mixes with xylol.

A number of mounting fluids were tried in an attempt to overcome this difficulty. The first material was glycerine jelly, which hardens and thus does not intermingle with the fresh balsam. But glycerine jelly proved unsatisfactory for so many of the mounts that it was discarded. Lacto-phenol was tried with even less success. It causes hyaline conidia later to show color, and destroys the sharpness of outline which is so much desired when studying colorless fungi.

Finally B. O. Dodge recommended Shear's mounting fluid and outlined a general method of procedure. These directions, with further ideas copied from the systems used by David Linder of Harvard and W. G. Solheim of Wyoming, are the basis for the following recipe which is now being used:

1. Use of a No. 0, 22 mm. and a No. 2, 12 mm. cover glass. An 18 mm. cover glass may be substituted for the larger cover glass if desired.

[1] Diehl, W. W. An improved method for sealing microscopic mounts. Science **69**: 276. 1929.

2. Fungi or algae may be mounted entire. Sections of host tissue are cut with a razor, or bits of leaf macerated in heated KOH (5 per cent solution). In the last case, the material is rinsed in water and then heated in Shear's mounting fluid which consists of:

2 per cent potassium acetate (in water)	300 cc.
glycerine	120 cc.
95 per cent alcohol	180 cc.

3. The material is placed in a drop of Shear's mounting fluid on the 12 mm. cover glass. Care must be taken to orient the sections or macerations so that the fungous or algal parts will be correct for examination when the slide is finished. In mounting fungi it is desirable to add additional scrapings of spores that may have been lost in the maceration process. The mount is heated carefully over a microburner until most of the liquid has evaporated.
4. Place drop of pure glycerine on mount and heat slightly again. This is very important, for glycerine takes up water readily, and if not heated will produce a milky opaque film.
5. Turn the 12 mm. cover glass with the material and heated glycerine upside down onto the 22 mm. cover glass. Press down firmly. Wipe away all excess glycerine from margin of smaller cover glass.
6. Place a generous drop of medium heavy balsam on a microscope slide. Heat balsam gently, then place cover glasses with the 12 mm. one underneath onto it. After the balsam has spread far enough to seal the glycerine, the whole mount can be pressed down until the balsam exudes slightly from the edge of the larger cover glass.

This method of mounting has served in an excellent manner for mounting types of *Cercospora* and *Meliola,* and for the study of *Venturia inaequalis.* It also has been employed by algologists, and by teachers, who wished to have permanent slides of stomatal arrangement on leaf tissue.—CHARLES CHUPP.

A New Cercospora from Oklahoma

In August and September of 1939, a species of *Cercospora* causing a serious leaf-spot of the leguminous plant, *Laburnum anagyroides* Medic., was collected in the gardens of the Agricultural and Mechanical College at Stillwater, Oklahoma. A brief search of the literature failed to disclose any species of *Cercospora* affecting this host. Specimens were sent to Dr. C. C. Chupp, who concluded that the fungus was undescribed. At his suggestion the writer offers the following description and name for the new species:

Cercospora Laburni sp. nov.

Maculae suborbiculares vel angularibus, 1–5 mm. diametro, cinereis, marginibus angustis atrorubrobrunneis; fungus stratum amphigenum; stromatis levis vel 50 μ crassis; plerumque in dense fasciculatis, saepe coremoideis, in masse nigeris, singulatim dilute olivaceo-brunneis, uniformis in coloris et latitutinis, parce septatis, non-ramosis, nonnumquam semel vel bis geniculatis, ad apices subtruncatis, 4–6 × 20–125 μ; conidiis hyalinis, acicularis, rectis vel nonnihil curvatis, septis inconspicuis, ad bases truncatis, ad apices acutis vel subacutis, 2–3.5 × 20–110 μ.

Hab. in foliis *Laburnum anagyroides.*

Leaf lesions subcircular to angular, inclined to coalesce, 1 to 8 mm. in length, gray to white center with a dark reddish-brown, narrow margin; fruiting amphigenous; stroma slight to 50 μ in width; fascicles mostly dense, often coremoid; conidiophores dark in mass, singly pale olivaceous-brown, uniform in color and width, sparingly septate, not branched, sometimes one or rarely twice geniculate, large spore scar at subtruncate tip, 4–6 × 20–125 μ; conidia hyaline, acicular, straight to slightly curved, septa indistinct, base truncate, tip acute to subacute, 2–3.5 × 20–110 μ.

Hab.: On leaves of *Laburnum anagyroides* in Stillwater, Oklahoma, August–September, 1939.

Type: In the herbarium of the Department of Plant Pathology, Cornell University, No. *28932*.—W. Winfield Ray.

Overwintered Giant Puff-Balls in Alberta

(with 1 figure)

In May, 1938, giant puff-balls, *Calvatia gigantea* (Batsch) Fries, were found at Edmonton, Alberta, on a north-facing slope domi-

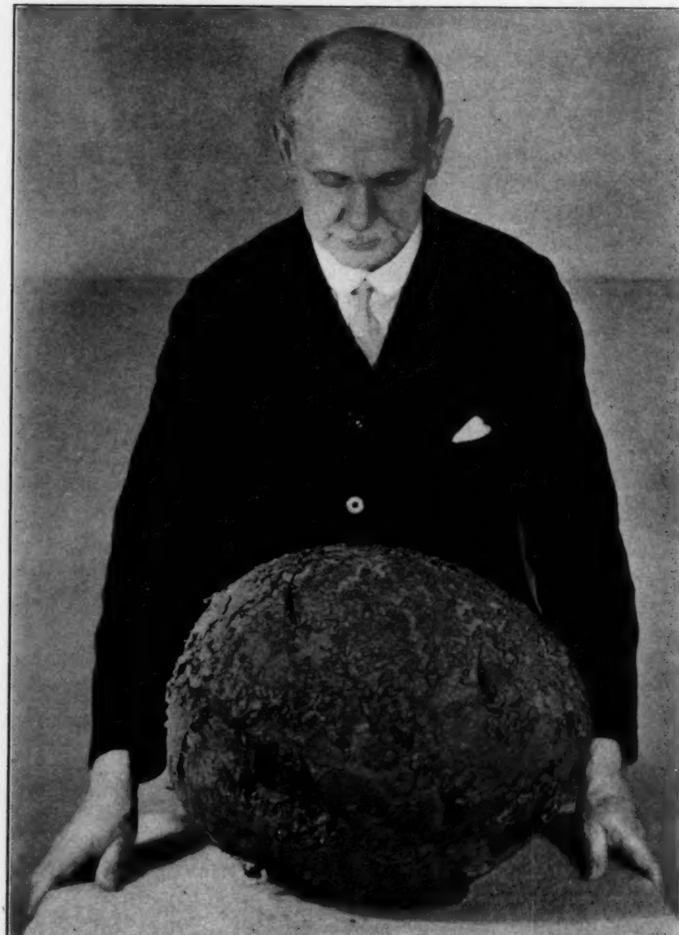

FIG. 1.

nated by grasses, shrubs and weeds. The largest of these puff-balls measured approximately 13 inches in height, 14 inches in diameter and 4 feet in circumference. Although undoubtedly fully matured the previous autumn, they were still in a good state of preservation when found on May 16th, their peridia being only

slightly broken and the spore masses mainly intact. The snow which covered them during the greater part of the winter disappeared in late March or early April, after which they were exposed to winds and, in early May, to heavy rains. Considerable protection against the elements was afforded by shrubs and by dead grass (*Calamagrostis, Bromus* and *Poa*) and nettles (*Urtica gracilis*) amongst which the puff-balls occurred.

On March 24th, 1939, the same area provided another collection of puff-balls most of which had overwintered in good condition. The largest of these (FIG. 1 [1]) measured 14 inches high, 14 inches wide, 16⅝ inches long and 50 inches in circumference, and weighed approximately 715 gms. after air-drying in the laboratory. At the time of collection, this puff-ball was water-soaked, the surrounding snow having only recently melted. In the laboratory the weight became more or less constant after sixteen days, fluctuating between 708 and 722 gms. and varying strikingly with the relative humidity of the room. Perhaps a very sensitive hygroscope might be made out of puff-ball gleba or capillitium system. The characters of the peridium show clearly in the photograph, especially the thin, fragile structure of the outer peridium which peels off in small, irregular patches. Buller [2] calculated that a giant puff-ball weighing 232 gms. contained about 7,000,000,000,000 spores. Therefore it may be estimated that our specimen, weighing 715 gms. contains over 20,000,000,000,000 spores.—E. H. Moss.

[1] Professor A. H. R. Buller, who was a visitor at the University of Alberta shortly after the puff-ball was found, appeared in this photograph at the writer's request.

[2] Researches on Fungi, I, p. 85, 1909.